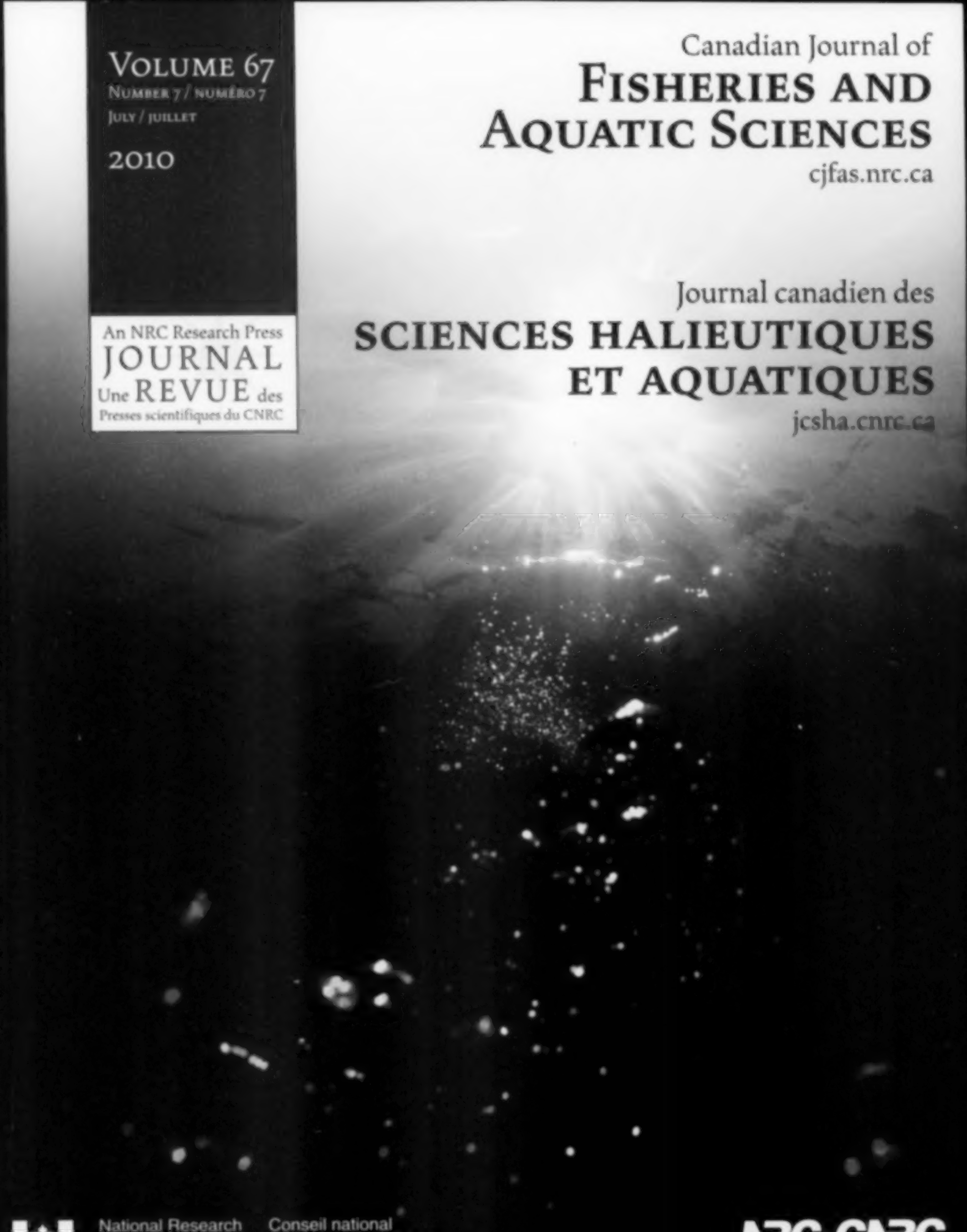

VOLUME 67
NUMBER 7 / NUMÉRO 7
JULY / JUILLET
2010

Canadian Journal of
FISHERIES AND AQUATIC SCIENCES
cjfas.nrc.ca

Journal canadien des
SCIENCES HALIEUTIQUES ET AQUATIQUES
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An NRC Research Press
JOURNAL
Une REVUE des
Presses scientifiques du CNRC

National Research Council Canada
Conseil national de recherches Canada

NRC·CNRC

ISSN 0706-652X / CJFSDX 67(7) 1057–1204 (2010)

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Volume 67, Number 7, July 2010

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 67, numéro 7, juillet 2010

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

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Consistent patterns of maturity and density-dependent growth among populations of walleye (*Sander vitreus*): application of the growing degree-day metric

Paul A. Venturelli, Nigel P. Lester, Terry R. Marshall, and Brian J. Shuter

Abstract: Growing degree-days (GDD, °C-days) are an index of ambient thermal energy that relates directly to an ectotherm's cumulative metabolism but is rarely used to describe growth and development in fish. We applied GDD to length and maturity data from 416 populations of walleye (*Sander vitreus*) from Ontario and Quebec, Canada (mean annual GDD = 1200 to 2300 °C-days). On average, males matured after they had experienced 6900 °C-days and reached 350 mm total length (L) (n = 77 populations), and females matured after 10 000 °C-days and at 450 mm L (n = 70). Across 143 populations, GDD accounted for up to 96% of the variation in the length of immature walleye but also revealed a twofold difference in growth rate that was indicative of variation in food availability. When applied to data from eight populations in which walleye abundances have changed dramatically over time, GDD revealed a 1.3-fold increase in immature growth rate when abundance was low compared with when it was high. Our results both demonstrate the explanatory power of GDD with respect to fish growth and maturity and inform the development of regional management strategies for walleye.

Résumé : Bien que les degrés-jours de croissance (GDD, °C-jours) soient un indice d'énergie thermale directement relié au cumulatif métabolisme chez les ectothermes, cet indicateur a rarement été considéré pour décrire la croissance et le développement des poissons. Nous avons utilisé l'indice GDD dans l'analyse de données de taille et de maturité provenant de 416 populations de dorés jaunes (*Sander vitreus*) de l'Ontario et du Québec, Canada (moyenne annuelle GDD = 1200 à 2300 °C-jours). En moyenne, les mâles ont atteint la maturité à 6900 °C-jours, correspondant à une longueur totale de 350 mm (n = 77 populations), et les femelles à 10 000 °C-jours, correspondant à une longueur totale de 450 mm (n = 70). Dans une analyse comprenant 143 populations, l'indicateur GDD a permis d'expliquer jusqu'à 96 % de la variabilité dans la taille des individus immatures, en plus de révéler que la variabilité dans l'abondance des proies peut conduire à des différences du taux de croissance allant jusqu'au double. En utilisant les données de huit populations au sein desquelles une grande variation de l'abondance des dorés avait vraisemblablement causé de grands changements dans l'abondance des proies, l'indice GDD a montré que le taux de croissance des individus immatures bénéficiant d'une forte disponibilité de proies était de 1.3 fois supérieur à celui d'individus subissant une faible disponibilité de proies. Nos résultats démontrent le pouvoir de l'indicateur GDD pour expliquer la variabilité dans la croissance et la maturité et seront utiles au développement de stratégies régionales de gestion des populations de dorés jaunes.

Introduction

Fish growth and development are primarily functions of food and temperature (Fry 1971; Kitchell et al. 1977; Paloheimo and Dickie 1966). Recently, Neuheimer and Taggart (2007) showed that growing degree-days (GDD, °C-days; an index of ambient thermal energy that relates directly to an ectotherm's cumulative metabolism but is rarely used in

Received 3 March 2009. Accepted 12 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 11 June 2010.
J21092

Paper handled by Associate Editor Marc Trudel.

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Can. J. Fish. Aquat. Sci. 67: 1057–1067 (2010)   doi:10.1139/F10-041   Published by NRC Research Press

fish science) could account for much of the variation in length ($r^2 \geq 0.92$) among species and populations of fish.

A corollary of the GDD approach is that variation in growth, over and above that associated with variation in GDD, may be attributable to factors other than temperature (e.g., food availability; Neuheimer and Taggart 2007). For decades, fisheries scientists at the Ontario Ministry of Natural Resources (OMNR), Canada, have used GDD to describe fish length and maturity (e.g., Colby and Nepszy 1981). Although Neuheimer and Taggart (2007) found that a single GDD function was sufficient to explain 93% of the variation in immature length among 17 stocks of Atlantic cod (*Gadus morhua*), we hypothesize that when both GDD and density vary strongly over time or across space, GDD will be associated with a broad range of immature lengths. Immature growth rate and length are important determinants of early survival (see reviews by Cowan et al. (2000), Miller et al. (1988), and Sogard (1997)) and significant life history parameters (e.g., age at maturity, size at age, adult size and longevity; Beverton and Holt 1959). Therefore, quantifying the temperature-independent effect of density on the growth of immature fish across numerous populations is important to understanding the reproductive biology and population dynamics of harvestable species that are distributed over large spatial scales.

In this study, we respond to Neuheimer and Taggart's (2007, p. 384) challenge to "test the GDD metric in all aspects of fish and aquatic invertebrate physiology, growth, and development" by first showing that GDD is a strong predictor of the length and maturity of walleye (*Sander vitreus*) — an economically important freshwater species that is a research and management priority throughout much of its North American range. Using data from eight populations in which walleye abundances have changed dramatically over time, we then use the GDD approach in a combined within- and among-population analysis to quantify a general effect of density on immature growth rate.

Materials and methods

Survey data

Walleye data were obtained from 416 water bodies in Ontario and Quebec (44°N to 53°N latitude). These water bodies were surveyed at least once in fall (surface water 10–15 °C; Morgan 2002) between 1988 and 2002 by either the OMNR or the Ministère des Ressources Naturelles et de la Faune du Québec. Typically, each survey represented eight or more depth-stratified, random, overnight sets of multimesh gill nets (for details, see Morgan 2002). Walleye total length (*L*), maturity, and sex were determined in the field, and age at capture was assigned in the lab using otoliths.

For each walleye population in Ontario, we generated a unique time series of annual growing degree-days ($\mathrm{GDD_a}$) above an air temperature threshold of 5 °C. We based $\mathrm{GDD_a}$ on air temperature because water temperature data are not broadly available and because walleye prefer to be above the thermocline (20–25 °C; Colby et al. 1979), where air and water temperatures are strongly correlated over a broad range of lake morphologies (Livingstone and Lotter 1998; Livingstone and Padisak 2007; Shuter et al. 1983). We used 5 °C as a base temperature because (*i*) it defines the walleye growing season in terms of both ice cover (Shuter et al. 1983) and bioenergetics (Kitchell et al. 1977), and (*ii*) an exploratory analysis following Neuheimer and Taggart (2007, see supplementary information) showed that the coefficient of determination of the relation between walleye length and the cumulative annual growing degree-days ($\mathrm{GDD_c}$) that each walleye experienced prior to capture was maximized for temperature thresholds in the range of 0 °C to 10 °C (Supplemental Fig. S1[4]).

To calculate $\mathrm{GDD_a}$ for a given population and year of interest, we (*i*) obtained daily air temperature data from all available Ontario weather stations for that year, (*ii*) calculated a $\mathrm{GDD_a}$ for each station (i.e., the difference between the average daily air temperature and 5 °C, summed across all days for which the average daily air temperature was ≥5 °C), (*iii*) GIS-interpolated these data to produce a province-wide 1 km^2 $\mathrm{GDD_a}$ grid map, and then (*iv*) assigned a unique $\mathrm{GDD_a}$ value according to the population's geographic coordinates. Because grid maps of $\mathrm{GDD_a}$ were unavailable for Quebec, we estimated the $\mathrm{GDD_a}$ for a given population and year directly from nearby temperature stations (i.e., using steps *i* and *ii* above; mean station distance to a lake = 43 km, range 2 to 173 km).

To calculate the $\mathrm{GDD_c}$ that each walleye experienced prior to capture, we summed population-specific values of $\mathrm{GDD_a}$ across all years in which that walleye was alive. Thus, a 2-year-old walleye from Jessica Lake in fall 2001 experienced three growing seasons (1999, 2000, 2001) prior to capture, each with a specific $\mathrm{GDD_a}$ value (1691, 1646, and 1817 °C·days, respectively) that summed to a $\mathrm{GDD_c}$ of 5154 °C·days. Because each $\mathrm{GDD_a}$ value in a time series was unique, a 2-year-old walleye from the same population but a different year would have experienced a different $\mathrm{GDD_c}$. (A summary of water body (location, surface area, and mean depth), temperature ($\mathrm{GDD_a}$ and $\mathrm{GDD_c}$), and walleye data (sex, age, *L*, and maturity) is provided in online Supplemental Table S1[4])

Length, maturity, and $\mathrm{GDD_c}$

The first step in our analysis of survey data was to define a threshold value of $\mathrm{GDD_c}$ below which both males and females were likely to be immature. This step was necessary because the allocation of surplus energy towards reproduction (Day and Taylor 1997; Lester et al. 2004) is likely to produce adult- and sex-specific relations between *L* and $\mathrm{GDD_c}$, and because the point at which walleye in our final analysis matured was unknown (see Immature length, $\mathrm{GDD_c}$, and density). We used two different methods to define this GDD-at-maturity threshold. The first method was based on male and female *L* at $\mathrm{GDD_c}$. It assumed that if both sexes grow similarly before maturity but differently after (Day and Taylor 1997; Lester et al. 2004), then deviation from a common *L*-at-$\mathrm{GDD_c}$ pattern should indicate maturity in one or both sexes. This method is useful when maturity data are absent. To estimate maturity thresholds from length data, we first calculated the age- and year-specific mean lengths for both males and females in each population ($n \geq 5$ individuals per mean); associated GDD_c values were calculated accordingly. For both sexes separately, we then fitted a cubic regression spline (Schluter 1988) to the relation between mean L and GDD_c. Splines were fit using a smoothing parameter that minimized prediction error; the 95% confidence interval (CI) for the spline fit was estimated by bootstrapping the data n times (n = 1629 and 1382 for males and females, respectively). We then defined GDD_c and L at maturity as the point at which the 95% CIs around male and female regression splines ceased to overlap.

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[4] Supplementary data for this article are available on the Journal web site (http://cjfas.nrc.ca).

The second method for estimating maturity thresholds was based directly on survey maturity data; this method also served as a check of the accuracy of the first, length-based approach. For each population, we estimated the L and GDD_c at 50% maturity (L_{50} and GDD_{50}, respectively) by probit analysis (Finney 1971; Trippel and Harvey 1991) of the frequency distribution of mature and immature walleye (n = 100 males or females pooled across all sample years within a population). We used 5 mm length bins when estimating L_{50}; because individuals of a given cohort and age shared a common thermal history (i.e., GDD_c), binning was not necessary when estimating GDD_{50}. Finally, we estimated maturity thresholds for both sexes by averaging L_{50} and GDD_{50} estimates across all populations. To further explore the predictive power of GDD with respect to maturity, we regressed values of L_{50} and age at 50% maturity (t_{50}) in each lake against mean annual growing degree-days ($\overline{\text{GDD}}_a$). Age at 50% maturity was estimated as above.

Immature length and GDD_c

Once we had defined a threshold value of GDD_c below which individual walleye were likely to be immature, we set out to determine how best to describe immature length. Specifically, we were interested in whether GDD_c explained more variation in immature length than age, and whether simple models were better than complex ones. Although Neuheimer and Taggart (2007) found that a single GDD_c function explained 93% of the variation in immature length among 17 stocks of Atlantic cod, residual variation in relations between immature length and GDD_c among surveyed walleye populations might be large enough to justify a more complex model. If total length is defined as

$$(1) \quad L = L_0 + a \cdot \text{GDD}_c^b$$

where a is a parameter ≥ 0 that depends on the energy that is available for growth, the exponent b is the rate at which L changes with GDD_c, and L_0 is total length at the beginning of the life history stage of interest (e.g., free swimming, exogenous feeding, piscivory, maturity), then the increase in total length (L') over the period used to derive GDD_c is given by

$$(2) \quad L' = a \cdot \text{GDD}_c^b$$

or

$$(3) \quad \log_{10} L' = \log_{10} a + b \cdot \log_{10} \text{GDD}_c$$

Note that $L' = L - L_0$. Equation 3 is ideally suited to comparative analyses of length data because (*i*) it allows for both linear and nonlinear growth trajectories (Lester et al. 2004; Quince et al. 2008), (*ii*) it implies a common temperature effect when slopes are consistent, and (*iii*) it implies a surplus energy effect when intercepts vary. For our analysis, we set L_0 to 40 mm, the approximate L at which young walleye become piscivorous (reviewed by Galarowicz and Wahl 2005) — with attendant changes in physiology (Brackevelt et al. 1989) and behaviour (Houde and Forney 1970) — and develop ventral scales (Colby et al. 1979).

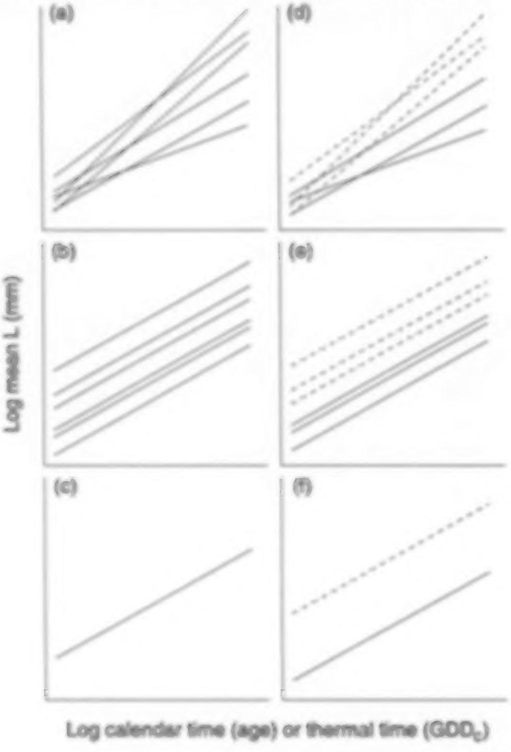
Our analysis proceeded as follows. First, we used the threshold value of GDD_c to extract from the survey data all individual walleye that were likely to be immature. We then calculated age- and year-specific mean lengths ($n \geq 5$ individuals, sexes combined) for immature walleye in each population; associated values of GDD_c were calculated accordingly. If a population was sampled more than once between 1988 and 2002 (31 of 143 populations in this analysis, only 12 of which were sampled over a period >5 years), mean values were calculated separately for each sampling year and then pooled under the assumption that walleye abundance had not changed dramatically between sampling years. Pooling in this way ensured that each population had equal weight in the subsequent analysis. We then used linear analysis of covariance (ANCOVA) of log-transformed data to develop three models of L' for both predictor variables (GDD_c and age): (*i*) a complex model in which a unique intercept and unique slope were estimated for each population (Fig. 1*a*), (*ii*) a model of intermediate complexity in which a unique intercept but a common slope were estimated for each population (Fig. 1*b*), and (*iii*) a simple model in which a common intercept and slope were estimated for all populations (Fig. 1*c*). To compare the strength of evidence for each of these six models, we then used a small-sample, bias-corrected form of Akaike's information criterion (AIC_c) to calculate model probabilities (w_i) (Anderson 2008; Burnham and Anderson 2002).

Immature length, GDD_c, and density

The purpose of our final analysis was to determine the efficacy of the GDD metric for quantifying an effect of density on immature length among numerous populations of walleye. For this analysis, we focused on eight well-studied populations in which walleye abundances changed at least fourfold as a result of overexploitation, experimental overexploitation, invasive species, and (or) recruitment failure stemming from introduced species, pollution, or the degradation of spawning habitat (Table 1; Supplemental Table S2[4]). We used either estimates or indices of walleye abundance, together with published accounts of abundance change, to divide data into periods of time when walleye densities were likely to be either relatively high or low. As described in the following paragraphs, we then used ANCOVA to compare immature L' at GDD_c both between density periods and among populations.

We estimated the L at age of individual walleye in each population and year primarily from the back-calculation of scales (Supplemental Tables S2 and S3[4]). We used back-calculation because gear size selectivity among young age groups can bias direct measurements of L towards fast-growing individuals. Back-calculations followed the Mon-

Fig. 1. Hypothetical examples of the linear analysis of covariance (ANCOVA) models that were evaluated in this study. In our analysis of survey data from 416 populations, we compared among the following six models: (*a*) log(mean total length (*L*)) vs. log(calendar time (age)) and log *L* vs. log(thermal time (GDD_c)), both with the categorical variable "population"; (*b*) log *L* vs. log age and log *L* vs. log GDD_c, both with no "population × time" interaction term; and (*c*) log *L* vs. log age and log *L* vs. log GDD_c, both with no categorical variable. In our analysis of eight collapsed populations, we compared among the following six models: (*a*), (*b*), and (*c*) with only log GDD_c as a covariate; (*d*) log *L* vs. log GDD_c with the categorical variables "population" and "density"; (*e*) log *L* vs. log GDD_c with the categorical variables "population" and "density" but no interaction terms; and (*f*) log *L* vs. log GDD_c with "density" as a categorical variable but no interaction terms. Continuous and broken lines in models (*d*), (*e*), and (*f*) illustrate change in *L* during periods of high and low density, respectively.



astyrsky nonlinear method (see Francis 1990). We derived the requisite slope of the relation between body length and scale size via a year-specific, log–log regression of *L* on anterior scale radius. If data from more than one year were available to estimate this slope, we used ANCOVA to calculate a common slope. Because confidence in scale ages tends to be low for older fish, we only back-calculated *L* at age using scales from 4-, 5-, and 6-year-old walleye (sexes combined). For each age and year, our minimum sample size was five fish per age class (e.g., *L* at age 1 in 1974 was derived from $n \geq 5$ age-4 walleye that were captured in 1977). When more than one age class contributed to back-calculated *L* in a year (e.g., both 4- and 5-year-olds were available to back-calculate *L* at age 1 in 1974), we used a Monte Carlo randomization procedure (up to 10 000 combinations) to test the null hypothesis of no difference between mean *L* at age. If this null hypothesis was rejected (19 of 171 cases in this study), we based *L* at age on the youngest age class; otherwise, data were pooled. Finally, we averaged across individual walleye within each year to produce annual estimates of *L* at age.

Some estimates of *L* at age were obtained from the literature as either back-calculations or direct measures (Supplemental Table S2[4]). Estimates were only included if $n \geq 5$ scales (or walleye) per population and year (sexes combined); however, data were not always available to limit back-calculations to scales from 4-, 5-, and 6-year-olds. When necessary, we developed population-specific regression equations to convert fork length to total length.

We obtained GDD_a and GDD_c values for populations in Ontario as described in Survey data. Missing GDD_a values in a time series were either estimated by linear regression of GIS-interpolated data onto data from the nearest weather station or obtained from the literature. For Saginaw Bay, Lake Huron, we estimated GDD_a and GDD_c directly from the nearest weather station (Supplemental Table S2[4]).

To test for an effect of density on length across all populations, we first divided each time series into periods of relatively high and low abundance. Analysis of *L* data then followed the combined ANCOVA–AICc approach that is described in the previous section, with two important changes. First, we excluded all age-based models by assuming a priori that GDD_c was better than age at explaining variation in *L*′ (recall that $L' = L - 40$ mm). This change reduced to three our original list of linear ANCOVA models: one complex (Fig. 1*a*), one intermediate (Fig. 1*b*), and one simple (Fig. 1*c*) relation between *L*′ and GDD_c, each with population as a categorical variable. Second, with the addition of abundance (high or low) as a categorical variable, we were able to evaluate three additional linear ANCOVA models: (*i*) a complex model in which a unique intercept and a unique slope were estimated for each population in both periods of abundance (Fig. 1*d*), (*ii*) a model of intermediate complexity in which a unique intercept but a common slope were estimated for each population in both periods of abundance (Fig. 1*e*), and (*iii*) a simple model in which a common intercept and a common slope were estimated for all populations in both periods of abundance (Fig. 1*f*).

Results

GDD_c and maturity

Cubic spline regression of 26 324 males from 1629 age classes across 369 populations in Ontario and Quebec indicated an abrupt change in *L* at GDD_c at ~6000 °C-days and ~340 mm *L* (Fig. 2*a*). The change in the *L* at GDD_c of females was less abrupt (22 077 individuals from 1382 age classes across 341 populations) but was first noticeable at ~7300 °C-days and ~380 mm *L* (Fig. 2*b*). When overlaid (Fig. 2*c*), these regression splines showed that the *L* at GDD_c of males and females was similar prior to maturation, but that the *L* at GDD_c of adults was sexually dimorphic.

Table 1. Populations used to relate the immature length of walleye to GDD_c (°C·days) at both high and low numerical abundance.

Population	ID	Location (latitude, longitude)	Area (ha)	Mean depth (m)	Magnitude of abundance decline[a]	Principal cause(s) of abundance decline
Crooked Pine Lake	1	48°47′N, 91°05′W	1 604	6.1	10.43	Recruitment failure via a species introduction
Lake Erie (west and west-central basins)	2	41°49′N, 82°38′W	1.95×10^6	16.6	3319.28	Overexploitation; recruitment failure
Henderson Lake	3	48°49′N, 90°18′W	151	2.5	15.44	Experimental overexploitation
Lake Huron (Saginaw Bay)	4	43°59′N, 83°29′W	2.96×10^5	10.1	18.45[b]	Recruitment failure via spawning habitat degradation
Lower Marmion Lake	5	48°53′N, 91°31′W	3 982	6.8	6.04	Recruitment failure via spawning habitat degradation
Lake Ontario (Bay of Quinte)	6	44°05′N, 77°05′W	25 740	10.0	2008.17	Overexploitation; recruitment failure
Savanne Lake	7	48°50′N, 90°06′W	364	2.6	4.47	Experimental overexploitation
Shoal Lake	8	49°33′N, 95°01′W	25 856	9.1	17.52[b]	Overexploitation

Note: See Supplemental Table S2 (available on the Journal web site (http://cjfas.nrc.ca)) for source information.

[a] Decline in abundance, expressed as the ratio of high to low abundance.

[b] Ratio based on commercial yield data.

Regression splines diverged at approximately 5900 °C·days and 340 mm L, and 95% CI diverged at approximately 6900 °C·days and 370 mm L (Fig. 2*d*). Estimates of GDD_c and L at maturity based on available maturity data tended to be larger than those based on L–GDD_c profiles, especially for females: on average, males matured at 6872 °C·days ± 457 95% CI and 349 ± 9 mm L, and females matured at 100037 ± 451 °C·days and 450 ± 10 mm L (Fig. 3). In terms of elucidating the maturity status of walleye in our eight study populations, we were confident based on these results that mean lengths associated with GDD_c values < 6000 °C·days were not confounded by the allocation of energy towards reproduction.

$\overline{GDD}_a$ (i.e., the length of an average growing season) did not explain more than 3% of the variation in L_{50} (Fig. 4*a*). However, we found relatively strong support for a negative relation between t_{50} and $\overline{GDD}_a$ (Fig. 4*b*).

Immature length and GDD_c

Across 143 populations and 9° of latitude (mean GDD_a = 1200 to 2300 °C·days), variation in log L' (i.e., L – 40 mm) of walleye that had experienced <6000 °C·days was best explained by the intermediately complex linear ANCOVA model based on log GDD_c (Table 2; Fig. 5*a*). However, even when temperature was taken into account, the L at GDD_c of immature walleye from different populations ranged between a low of $\log_{10} L' = 0.81 \cdot \log_{10} GDD_c - 0.75$ and a high of $\log_{10} L' = 0.81 \cdot \log_{10} GDD_c - 0.38$), a more than twofold difference in L at GDD_c (Fig. 5*a*). Empirical support for the remaining five models was negligible; however, the intermediately complex models always outperformed the simple models, which themselves outperformed the complex models (Table 2). Within this hierarchy, log GDD_c was consistently better than log age at explaining variation in log L'. The simple log GDD_c model (which was similar to the simple, untransformed GDD_c models presented in Neuheimer and Taggart 2007) was ranked third (Table 2) and took the form $\log_{10} L' = 0.78 \cdot \log_{10} GDD_c - 0.45$. For comparative purposes, a plot of log L' versus log age is shown (Fig. 5*b*).

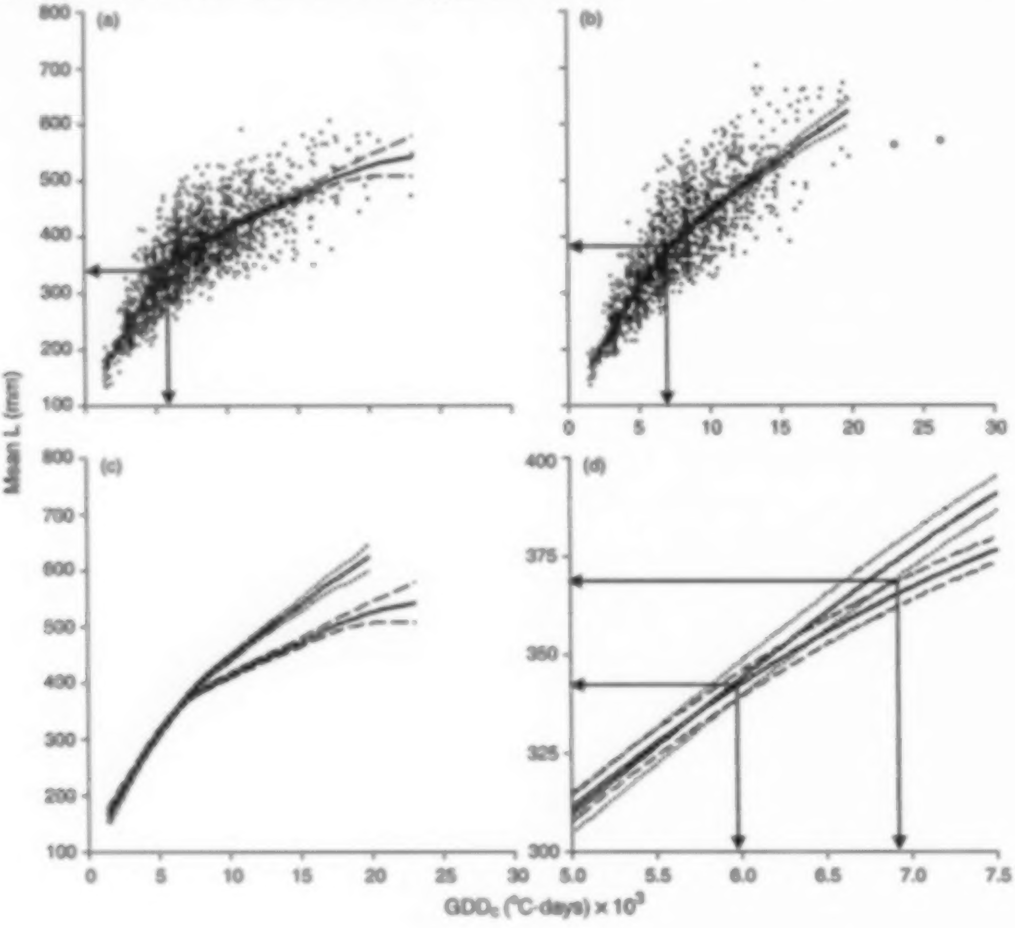
Immature length, GDD_c, and density

For the eight populations in which walleye abundances changed dramatically over time, empirical support was strongest for the intermediately complex linear ANCOVA model, with a common slope for log GDD_c but intercept values that were dependent on abundance (Table 3; Fig. 1*e*). In other words, although the L-at-GDD_c patterns of immature walleye varied among populations, they responded similarly to changes in abundance. On average, the L at GDD_c of immature walleye was described as $\log_{10} L' = 1.03 \cdot \log_{10} GDD_c - 1.43$ when abundances were high and then increased to $\log_{10} L' = 1.03 \cdot \log_{10} GDD_c - 1.32$ when abundances were low (a 1.3-fold increase; Fig. 6*a*). Empirical support for the remaining five models was negligible, although models that included the categorical variable "density" tended to outperform models that ignored it (Table 3). The simple log GDD_c model was ranked last (Table 3) and took the form $\log_{10} L' = 1.03 \cdot \log_{10} GDD_c - 1.39$ (Fig. 6*b*).

Discussion

Our results demonstrate the efficacy of GDD as a metric for explaining length in fishes (see also Neuheimer and Taggart 2007). We attribute the consistency with which GDD models outperform age-based equivalents to two factors. First, because age data are discrete (age-1, age-2, age-3, etc.), age-based models severely limit the distribution of L data along the x axis. For example, when age was used in place of GDD_c in Fig. 4, length data were restricted to five columns, one for each observed age. This change to the structure of the independent variable represents a considerable loss of information with which to explain variation in

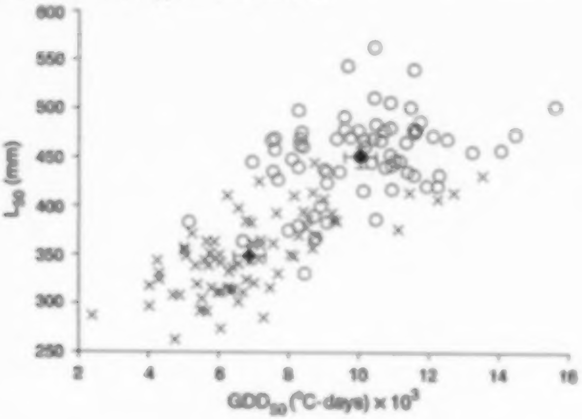
Fig. 2. Mean total length (L) versus the cumulative annual growing degree-days (GDD_c, °C·days) for (*a*) male (n = 1629 age classes from 369 populations) and (*b*) female walleye (n = 1382 age classes from 341 populations) in Ontario and Quebec. (*c*) and (*d*) Male and female regression lines (*c*) over the full range of total length (L) and GDD_c values and (*d*) at the point of divergence. Continuous lines are cubic splines (Schluter 1988), and broken and dotted lines are bootstrapped 95% confidence interval (CI) for males and females, respectively. Open circles in (*b*) were excluded from analysis. Arrows indicate the GDD_c and L at which splines appeared to bend (*a* and *b*) or 95% CI ceased to overlap (*d*) early in life. Data are available from Supplemental Table S1[4].



the dependent variable (Cottingham et al. 2005). Second, L–age relations ignore the fact that aging and growing are distinct processes insofar as a fish that survives over some time interval will age but the extent to which it increases in L depends not only on time, but also on other factors such as temperature. Put another way, unless the thermal energy (e.g., GDD) that fish are experiencing is known, using age to predict when a fish will reach a certain L is as effective as using a stopwatch alone to predict when a pot of water will boil.

Though GDD is clearly superior to age for describing immature length, our results also show that more complex GDD-based models can reveal patterns of L at GDD_c both through time and among populations. For example, our analysis of survey data from 143 populations of walleye suggests strongly that intercepts were population-specific and that changes in L' at GDD_c implied twofold variation in growth rate among populations. Our analysis of L data from eight populations also shows that relations between log L' and log GDD_c differed between periods of high and low abundance (i.e., density) within a population; however, the magnitude of this difference was relatively consistent across populations and suggested that immature walleye grew 1.3 times faster when population densities were low compared with when population densities were high. Neuheimer et al. (2008) also showed, in a population of haddock (*Melanogrammus aeglefinus*), that L–GDD_c relations showed a reduction in L at GDD with increasing population density (1965–1996; Frank et al. 2001) but proposed that these L changes resulted from a gradual removal of fast-growing and late-maturing individuals by size-selective fishing mortality. Given that fishing mortality in our eight study lakes was (*i*) relatively low or absent when densities were high, (*ii*) relatively high when densities were low, or (*iii*) altogether

Fig. 3. Total length at 50% maturity (L_{50}) and GDD at 50% maturity (GDD_{50}) of male (×, n = 77 populations) and female (○, n = 70 populations) walleye in Ontario and Quebec as determined by probit analysis. Solid symbols within each cluster of points are sex-specific means of L_{50} and GDD_{50} ± 95% confidence interval (CI). L_{50} increased with GDD_{50} across all populations because ectotherms must accrue thermal energy if they are to grow. Data are available from Supplemental Table S1.



negligible in shaping density patterns, size selectivity is insufficient to explain the observed increase in L at GDD_c in this study.

Density-dependent changes in L at age have been observed in many individual populations of walleye (e.g., Anthony and Jorgensen 1977; Colby and Nepszy 1981; Reid and Momot 1985); however, direct comparisons involving two or more populations are comparatively rare (a trend that is by no means restricted to walleye). To a large extent, individual case studies are common because of a need for detailed information on individual populations in which L at GDD_c is often the result of numerous factors and their interactions. However, it is also a symptom of the age-based approach to describing length, which limits the efficacy or even feasibility of among-population analyses by emphasizing, rather than accounting for, differences among populations. Length models based on GDD, on the other hand, explicitly account for temperature (arguably one of the most important determinants of growth; Fry 1971; Kitchell et al. 1977; Paloheimo and Dickie 1966) and, in doing so, simplify considerably the job of estimating how immature length changes with density. Furthermore, by relating length to a common currency (thermal age), the GDD metric facilitates the comparison or even aggregation of data across populations. For example, our results with respect to L' at GDD_c suggest a consistent, 1.3-fold increase in immature growth rate with large reductions in density, information that is useful for developing management strategies that can be generalized to multiple populations and (or) disturbance scenarios. Sass and Kitchell (2005) found that GDD_a contributed negligibly (partial R^2 = 0.001 for males and 0.02 for females) to a multiple regression model (R^2 = 0.34 for both sexes) that used seven predictor variables (including walleye density) to explain variation in walleye L across 859 lakes in Wisconsin. Given that this model included no other temperature variables, we suspect that GDD_a performed poorly in this case because it was estimated from a single, central location but then applied to an area that spanned 7.5 × 10^4 km^2 and 3° of latitude; ideally, a unique GDD_a time series should be developed for each lake. Furthermore, because they used GDD_a from the most recent growing season to explain variation in age-3 (males) and age-5 (females), these authors did not account for thermal energy that had accumulated in earlier growing seasons. To illustrate these points using our data, we used linear ANCOVA of age-3 L versus location-specific GDD_c with density as a categorical variable and then repeated this analysis using a single, central GDD_a time series (Sudbury, Ontario, Canada: 46°38′N, 80°48′W). Whereas the location-specific

Fig. 4. Linear regression of (a) total length at 50% maturity (L_{50}) and (b) age at 50% maturity (t_{50}) on mean annual growing degree-days ($\overline{GDD}_a$, °C-days) for males (×, broken lines) and females (○, continuous lines). Regression results are as follows: male L_{50} (r^2 = 0.03, n = 77, P = 0.132); female L_{50} ($r^2 < 0.01$, n = 70, P = 0.884); male $t_{50} = 61846 \cdot \overline{GDD}_a^{-1.306}$ (r^2 = 0.32, n = 77, $P < 0.0001$); female $t_{50} = 36666 \cdot \overline{GDD}_a^{-1.175}$ (r^2 = 0.42, n = 70, $P < 0.0001$). Data are available from Supplemental Table S1[4].

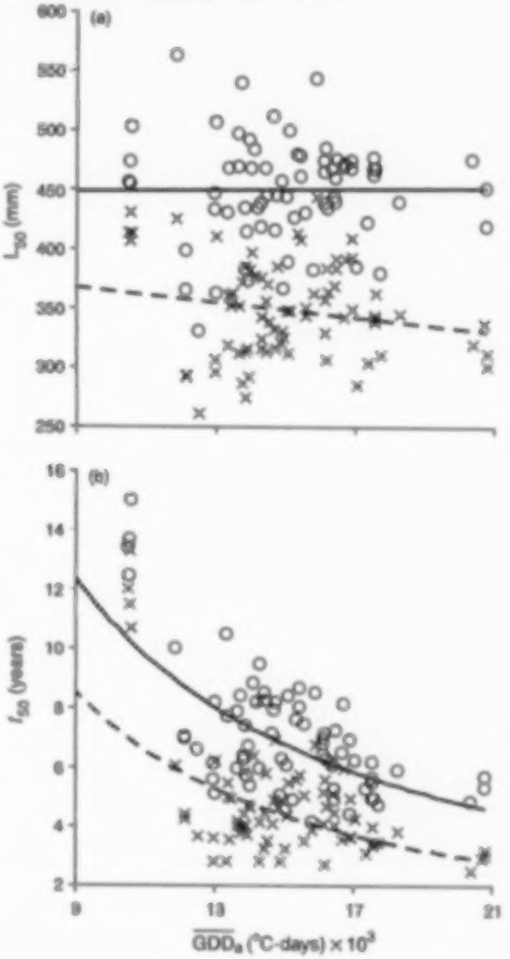


Table 2. The strength of evidence for six linear analysis of covariance (ANCOVA) models of log(immature total length (L')) according to data from 143 walleye populations in Ontario and Quebec.

Model	Predictor variable	Intercept	Slope	n	K	$\log(\hat{\sigma}^2)$	AIC_c	AIC_c rank	Δ_i	w_i	r^2_{adj}
b	log GDD_c	Unique	Common	637	145	−2.999	−1534.464	1	0.000	1.000	0.95
b	log age	Unique	Common	637	145	−2.926	−1487.643	2	46.821	<0.000	0.94
c	log GDD_c	Common	Common	637	3	−2.317	−1470.912	3	63.552	<0.000	0.82
c	log age	Common	Common	637	3	−2.113	−1339.879	4	194.585	<0.000	0.70
a	log GDD_c	Unique	Unique	637	287	−3.258	−1027.573	5	506.891	<0.000	0.96
a	log age	Unique	Unique	637	287	−3.157	−963.555	6	570.909	<0.000	0.95

Note: $L' = L - 40$ mm. Models correspond to panels in Fig. 1. Models were ranked according to strength of evidence as determined by a small-sample, bias-corrected form of Akaike's information criterion (AIC_c). n, sample size (number of data points across all populations); K, number of parameters (intercept(s) + slope(s) + error estimate); $\hat{\sigma}^2$, residual variance; Δ_i, AIC_c differences; w_i, Akaike weight; r^2_{adj}, adjusted coefficient of determination; GDD_c, cumulative annual growing degree-days.

Fig. 5. Linear analysis of covariance (ANCOVA) model of log-transformed (*a*) mean total length (L', mm) and (*b*) age (years) of immature walleye (GDD_c < 6000 °C·days, sexes combined) versus log-transformed cumulative annual growing degree-days (GDD_c, °C·days) in 143 surveyed populations. Broken and continuous lines represent the fastest and slowest predicted growth trajectories, respectively, according to model *b* (Fig. 1*b*); thin lines represent the 95% prediction intervals. Numeric symbols in (*a*) denote age at time of sampling in a population and year. Untransformed data are available from Supplemental Table S1[4].

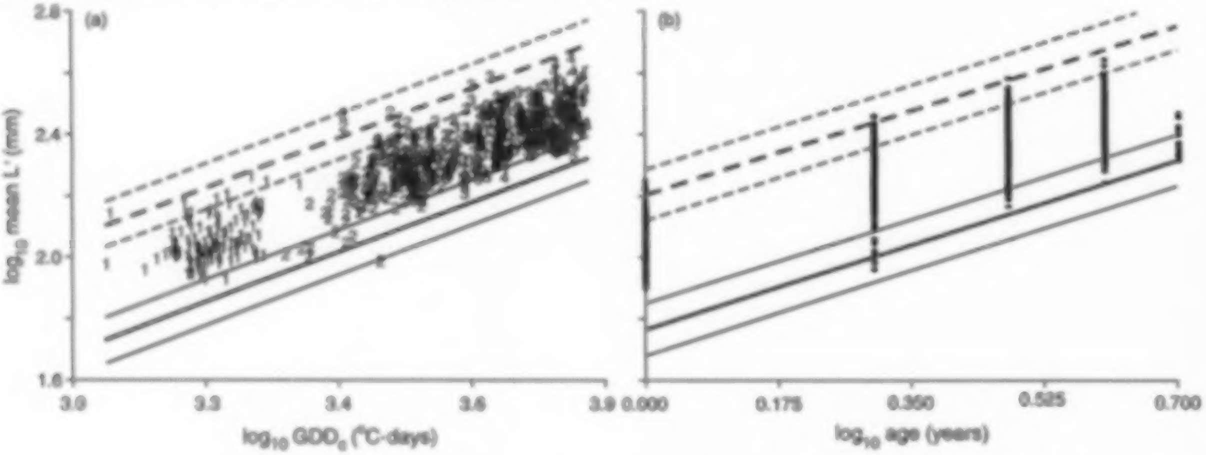


Table 3. The strength of evidence for six linear analysis of covariance (ANCOVA) models of log(immature total length (L')) versus log GDD_c according to data from eight walleye populations in which densities have changed dramatically over time.

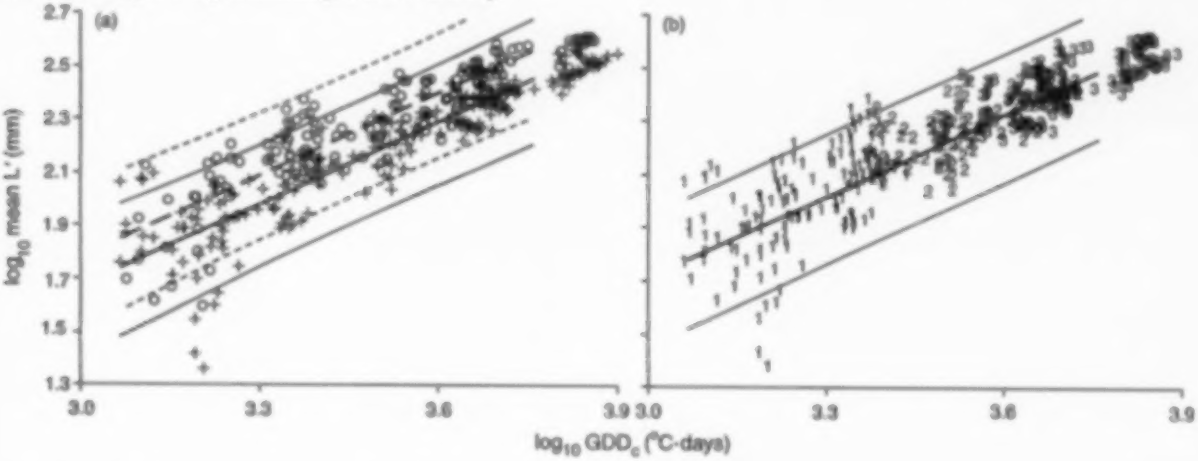
Model	Density	Intercept	Slope	n	K	$\log(\hat{\sigma}^2)$	AIC_c	AIC_c rank	Δ_i	w_i	r^2_{adj}
e	Included	Unique	Common	339	11	−2.481	−818.377	1	0.000	1.000	0.87
d	Included	Unique	Unique	339	33	−2.273	−697.369	2	121.008	<0.000	0.89
a	Ignored	Unique	Unique	339	17	−2.040	−655.807	3	162.570	<0.000	0.82
b	Ignored	Unique	Common	339	10	−1.980	−650.664	4	167.713	<0.000	0.80
f	Included	Common	Common	339	4	−1.943	−650.540	5	167.837	<0.000	0.78
c	Ignored	Common	Common	339	3	−1.844	−619.169	6	199.208	<0.000	0.73

Note: See Table 2 for comments and definitions. The terms "common" and "unique" apply within a density category for models *d* and *e*, but between density categories for model *f*.

GDD_c term in the first model was highly significant (P = 1.41 × 10^{-9}), the single-location GDD_s term in the second model was not (P = 0.06). Overall, our results suggest that when thermal history is accurately accounted for, length can be used as a surrogate method for monitoring large changes in abundance.

The density-dependent growth response that is implied by our results is not trivial. Because growth rate and size are critical to the early survival of fish (see reviews by Cowan et al. (2000), Miller et al. (1988), and Sogard (1997)), even small changes in growth can significantly affect both the timing and number of individuals that recruit into a fishery. For example, the GDD_s for Oneida Lake, New York, is 2180 °C·days (Goddard Institute for Space Studies 2008;

Fig. 6. Linear analysis of covariance (ANCOVA) models of log-transformed mean total length of immature walleye (L') (sexes combined) versus log-transformed cumulative annual growing degree-days (GDD_c, °C-days) for eight collapsed stocks with data (*a*) divided into periods of high (+ and continuous line) and low (○ and broken line) density (Fig. 1*e*; showing mean relations within a density period) and (*b*) pooled over the entire time series (Fig. 1*c*). Thin lines are 95% prediction intervals; 95% confidence intervals in (*a*) (not shown) did not overlap. Numeric symbols in (*b*) identify calendar ages in years. Points above the 6000 °C-days threshold were excluded from analysis because of the potentially confounding effects of maturity.



our calculation). If we assume that density-dependent growth responses follow Fig. 6*a*, then a GDD_a of 2180 °C-days predicts that 1- and 3-year-old walleye will grow to 133 mm and 330 mm *L*, respectively, when density is high, and 162 and 419 mm *L*, respectfully, when density is low. Given that walleye in Oneida Lake (*i*) can experience ~1.5 times more overwinter mortality at 133 mm *L* than at 162 mm *L* (Rose et al. 1999; our calculation), (*ii*) become vulnerable to the sport fishery at 381 mm (15 in.) *L*, and (*iii*) produce ~15 billion eggs·year^{-1} (Forney 1976), and also assuming that instantaneous rates of natural mortality follow Venturelli et al. (2010), these relatively small differences in predicted *L* at age translate into dramatic differences in both when and how many walleye are predicted to recruit into the fishery: three age-3 walleye when density is relatively high for every one age-4 walleye when density is relatively low. Conversely, this difference represents the extent to which recruitment can be over- or under-estimated if information on density-dependent immature growth is unavailable or ignored.

In addition to immature length, our results bear on the relevance of GDD to other aspects of walleye life history. Relations between *L* and GDD_c show clearly that adult lengths are sexually dimorphic over broad temporal and spatial scales (Henderson et al. 2003; Rennie et al. 2008; Sass and Kitchell 2005) and, together with relations between L_{50} and GDD_{50}, that females tend to mature later and at a larger size than males. This sexual dimorphism in both maturity and adult length is consistent with life history theory (Charnov 1993; Roff 1992), which predicts that the minimum size threshold that an individual fish must reach before the benefits of maturity outweigh the costs is lower in males than in females. Overall, our application of GDD to data from 416 populations between GDD_a of 1000 °C-days and 2200 °C-days shows clearly that most walleye delay maturation until they reach ~340 mm *L*. Thus, ~6000 °C-days represents a thermal constant: the thermal energy that walleye must experience to reach a maturation size threshold of ~340 mm *L*.

Furthermore, if the *L* at maturity of walleye and other long-lived species is relatively constant (Beverton 2002; Rochet 1998; this study), then the rate at which an individual can grow each year (i.e., $\overline{\mathrm{GDD}}_a$) should be a stronger predictor of t_{50} than L_{50} because growth rate determines the time that it takes to reach a size threshold, but not that size threshold itself (Roff 1991). Accordingly, we found no support for a relation between L_{50} and $\overline{\mathrm{GDD}}_a$ but strong support for a relation between t_{50} and $\overline{\mathrm{GDD}}_a$ (see also Baccante and Colby 1996). Thus, male and female walleye in our study matured at ~350 and ~450 mm *L*, respectively, but, depending on $\overline{\mathrm{GDD}}_a$, took 2–15 years to do so. The extent to which L_{50}, t_{50}, and their relations to GDD vary with walleye density and how this variation can shape reproductive rates will be addressed in a future publication.

In conclusion, we echo the call by Neuheimer and Taggart (2007) for further research into the GDD metric, the limited use of which in fish science is out of proportion with its demonstrated utility (e.g., Colby and Nepszy 1981; Neuheimer and Taggart 2007; Ridgway et al. 1991). Although the present study shows that GDD can be used to account for variation in temperature when explaining variation in growth and development, it also raises several questions. (*i*) Are *L*–GDD relations consistent throughout a species' tolerance limits or geographical range? Evidence from walleye suggests that the effects of temperature on growth vary among phylogeographic lineages (Zhao et al. 2008; see also Conover et al. 2006). (*ii*) Does GDD predict other aspects of life history? Because maturity depends on cumulative temperature, then perhaps GDD_c at maturity should be used in place of age at maturity. (*iii*) How does GDD influence predictions of growth and development under future climate scenarios or in other species? For example, the GDD metric might perform poorly for lake trout (*Salvelinus namaycush*), which seek out thermal refugia (Snucins and Gunn 1995).

We support the results of Neuheimer and Taggart (2007) by demonstrating the explanatory power of the GDD metric with respect to variation in maturity- and density-dependent *L* at age across hundreds of populations and over a large spatial area. We also expand upon this work by emphasizing the importance of identifying other factors that affect growth, to what extent, and under what circumstances. Although the GDD metric is by no means a panacea, this statistically powerful, "common currency" approach has major advantages over relations between length and calendar time, which must be developed separately for each temperature scenario (e.g., population, treatment, period of time) and generally complicate the synthesis of data from unrelated studies (e.g., through meta-analyses). Given these advantages and the relative ease with which GDD data can be obtained, we strongly encourage the widespread use of GDD in place of age when studying growth and development in fish.

Acknowledgements

This paper is dedicated to the memory of Mike Fruetel, whose shortened career provided early insight into walleye growth and thermal history. Walleye data from Ontario were collected by the Cooperative Freshwater Ecology Unit at Laurentian University and the Ontario Ministry of Natural Resources (OMNR) under the direction of George Morgan and with data assembly by Michael Malette. Walleye data from Quebec were collected and organized by Daniel Nadeau and staff at the Ministère des Ressources Naturelles et de la Faune du Québec. The authors are also grateful to the following individuals for sharing scale samples, data, and expertise: F. Amtstaetter, J. Casselman, B. Jackson, T. Johnson, T. Mosindy, Y. Zhao (all of the OMNR), and D. Fielder (Michigan Department of Natural Resources). The authors also thank A. Cho, L. Cruz-Font, and L. Wang for helping to back-calculate scales, J. Trumpickas for summarizing the Quebec temperature data, and D. Robert for translating the abstract. Earlier versions of this manuscript were improved by comments from P. Abrams, N. Collins, D. Hewitt, H. Rodd, C. Taggart, E. Trippel, S. Walker, and J. Ward and reviews by A. Neuheimer and an anonymous referee. This study was funded by the OMNR, the University of Toronto, and the Natural Sciences and Engineering Research Council of Canada.

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Population-specific locomotor phenotypes are displayed by barramundi, *Lates calcarifer*, in response to thermal stress

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Abstract: We investigated how thermal stress may alter the locomotor phenotype of barramundi, *Lates calcarifer*, from genetically distinct northern (low latitude) and southern (high latitude) populations in tropical Australia. Following early growth and development under native (25 °C and 30 °C) and non-native (20 °C and 35 °C) temperatures, we observed distinct differences in the swimming performance of northern and southern individuals that were consistent with expectations based on local thermal adaptation. Southern population fish exhibited significantly faster swimming speeds (32.10 ± 0.33 $\mathrm{cm\cdot s^{-1}}$, mass-adjusted mean ± 95% confidence limit) than their northern counterparts (28.58 ± 0.64 $\mathrm{cm\cdot s^{-1}}$) under cold-stress (20 °C) conditions. Conversely, northern population fish performed significantly better (51.63 ± 2.1 $\mathrm{cm\cdot s^{-1}}$) than their southern counterparts (44.18 ± 3.11 $\mathrm{cm\cdot s^{-1}}$) under heat-stress (35 °C) conditions. We conclude that *L. calcarifer* display locomotor phenotypes thermally adapted to their local environment, with early growth under non-native temperatures leading to significantly reduced phenotypic fitness.

Résumé : Nous étudions de quelle manière le stress thermique peut modifier le phénotype locomoteur du barramundi, *Lates calcarifer*, dans des populations génétiquement distinctes du nord (de basse latitude) et du sud (de haute latitude) d'Australie tropicale. Après le début de la croissance et du développement à des températures de leur milieu d'origine (25 °C et 30 °C) ou non (20 °C et 35 °C), nous observons de nettes différences dans la performance de nage entre les individus du nord et du sud qui correspondent aux attentes basées sur l'adaptation thermique locale. Les populations du sud ont des vitesses de nage significativement plus élevées (32,10 ± 0,33 $\mathrm{cm\cdot s^{-1}}$, moyenne ajustée à la masse ± intervalles de confiance de 95 %) que les populations du nord (28,58 ± 0,64 $\mathrm{cm\cdot s^{-1}}$) dans des conditions de stress au froid (20 °C). À l'inverse, les populations de poissons du nord performent significativement mieux (51,63 ± 2,1 $\mathrm{cm\cdot s^{-1}}$) que celles du sud (44,18 ± 3,11 $\mathrm{cm\cdot s^{-1}}$) dans des conditions de stress à la chaleur (35 °C). Nous concluons que *L. calcarifer* possède des phénotypes locomoteurs adaptés thermiquement à leur environnement local et qu'une croissance au début de la vie à des températures autres que celles de son milieu d'origine mène à une réduction significative de la fitness phénotypique.

[Traduit par la Rédaction]

Introduction

Organisms can respond via changes in their morphology, physiology, or survivorship to the temperature regime of their native habitat. Ectothermic, or cold-blooded, organisms are particularly responsive to their home-range temperatures due to the direct impact that temperature fluctuations (e.g., daily, seasonally, or climatically) have on their internal temperatures and metabolic function. Temperature differences are also known to manifest at the population level in ectothermic species through faster development, smaller body sizes, and shorter maturation times in warm-water vs. cold-water populations (i.e., "hotter is smaller" and "hotter is better" rules; Kingsolver and Huey 2008).

One physiological metric that has found great utility in the study of the phenotypic response of fishes to their environment is swimming performance. Swimming plays a central role in the lives of fishes, being involved in the essential tasks of acquiring food, avoiding predators, and finding mates (Batty and Domenici 2000; Blake 2004; Webb 1994). Swimming also constitutes one of the largest daily costs in the energy budget of fishes (Boisclair and Sirois 1993; Boisclair and Tang 1993; Feldmeth and Jenkins 1973) such that even small changes in swimming behaviour and perform-

Received 11 November 2009. Accepted 21 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 8 June 2010.
J21513

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Can. J. Fish. Aquat. Sci. 67: 1068–1074 (2010)   doi:10.1139/F10-047   Published by NRC Research Press

ance can have a major impact on the energy available for growth, repair, and reproduction. As such, examining the swimming performance of fishes can provide key insights into the factors that affect the health of individuals and populations, as well as evolutionary fitness (Huey and Stevenson 1979). Indeed, measures of swimming speed performance such as critical swimming speed performance (U_{crit}; Brett 1964) have provided important insights into how different temperature stresses are linked to variations in their health and survival (reviewed by Fulton 2010; Plaut 2001). Specific examples of these insights include how the environment has influenced the rate of early development of larval fishes (Fisher et al. 2000), attainment of food (Asaeda et al. 2005), avoidance of predators (Langerhans et al. 2004; Masuda 2006), outperformance of competing males and improved reproductive success (Kodric-Brown and Nicoletto 2005), migration to new habitats (Hinch and Rand 1998), and (or) competition for optimal locations within native habitats (Fulton et al. 2005; Kingsolver and Huey 2008). In many of these studies, the metric of U_{crit} has provided valuable insights into the health and fitness requirements of individuals (both inter- and intra-specific) in occupying particular environments.

In this study, we explore the physiological response of the Australian barramundi, *Lates calcarifer*, from two genetically discrete low and high latitude populations (Northern Territory and Central Queensland, respectively, Australia; Pusey et al. 2004) grown under thermal conditions outside their native annual water temperatures of 29 ± 4 °C (Rea et al. 2002) and 25 ± 3 °C (Simon Fisher, Queensland Department of Environment and Resources Management, Indooroopilly QLD 4068, Australia, personal communication), respectively. Barramundi is a catadromous hermaphrodite residing in rivers, estuaries, and shallow marine environments at different stages of its life cycle (Chenoweth et al. 1998; Keenan 1994). Moreover, *L. calcarifer* occurs throughout the Asian archipelago (13°N–10°S) and the rivers of northern Australia (25°S–12°S), with significant genetic partitioning between major Australian river catchments as indicated by microsatellite and allozyme analyses (Chenoweth et al. 1998; Doupé et al. 1999; Keenan 1994). Such genetic partitioning may indicate that each Barramundi population has accumulated unique adaptations to their native river environments leading to population-specific responses to environmental stress. Given that this species is successfully reared in captivity as part of the aquaculture industry, barramundi is also an ideal species for investigating how population-specific temperature responses may affect aquaculture outcomes.

In examining two distinct populations of *L. calcarifer*, we aimed to compare their phenotypic response after early growth under native, cold- and hot-stress thermal conditions corresponding to the minimum winter and maximum summer water temperatures across their range (20 °C and 35 °C, respectively). Using U_{crit} performance to describe their locomotor phenotype, we tested the hypotheses that (*i*) northern *L. calcarifer* individuals will perform significantly better than southern *L. calcarifer* individuals when both are grown under heat-stress (35 °C) conditions; (*ii*) southern *L. calcarifer* individuals will perform significantly better than northern *L. calcarifer* individuals under cold-stress (20 °C) conditions; and (*iii*) the swimming performance of southern *L. calcarifer* acclimated to cold-stress (20 °C) conditions will be significantly reduced when swum under non-acclimation temperatures (25, 30, and 35 °C).

Materials and methods

Study species

Juvenile *L. calcarifer* (approx. 90 days from hatching) were obtained directly from two hatcheries within Australia, the Darwin Aquaculture Centre (hereafter referred to as northern) and Kuranda Fish Farm (hereafter referred to as southern), which are located in the Northern Territory and North Queensland and source their broodstock from rivers in the Darwin (12°27.7′S, 130°50.5′E) and Bowen (20°0.7′S, 148°14.8′E) regions, respectively. The first batch of similar-sized fingerlings was obtained from the northern hatchery on 21 March 2007, with an average total length (TL) of 60.4 ± 0.4 mm. Fingerlings were air-freighted in slightly brackish water (5 ppt) at an average temperature of 26.7 °C ± 1 °C; zero mortality occurred during transportation. Fingerlings were immediately transferred to twelve 200 L aquaria (distributed at an equal density of 1 individual per 11.8 L) housed in temperature-controlled rooms at the Australian National University (ANU) Aquarium Facility in Canberra, Australian Capital Territory. Prior to the arrival of fingerlings, all tanks were set to a temperature of 30.0 ± 1 °C and a 14 h light – 10 h dark regime. Each tank's filtration was maintained by air-powered, biologically activated, under-gravel filters. Fingerlings were fed twice daily with a set quantity (approximately 4 g) of 1.5 mm pellets (Skretting Inc., Cambridge, Tasmania) per aquarium for the first 19 days, followed by 4 g of 2 mm pellets thereafter as per industry standard (Boonyaratpalin and Williams 2002). Minor amounts of food were left uneaten in all tanks (regardless of treatment), indicating that this level of food was enough for all fish to feed to satiation.

A second batch of *L. calcarifer* was obtained from the southern hatchery on 30 May 2007, with an average TL of 61.9 ± 0.4 mm. Fingerlings were air-freighted in slightly brackish water as before, but at an average temperature of 22.0 ± 1 °C; zero mortality occurred during transportation. As with the northern fingerlings, all tanks were set to a uniform 14 h light – 10 h dark regime, although initial tank temperatures were set to 25.0 ± 1 °C to match the lower initial temperature used in the hatchery and transportation of fingerlings. All southern fish were distributed among the same (unaltered) tanks at the same densities as for the northern fish and were, therefore, acclimated under the same conditions and feeding regime as the northern individuals.

Temperature treatments

Each temperature treatment (Table 1) was applied to three separate tanks, with a total of 51 fish per treatment, divided into three replicate groups of 17 fish each (except 20 °C treatment, n = 52). For the northern individuals, all tanks were held at 30.0 ± 1 °C for 4 days to allow for general acclimation. Deviation from the general acclimation temperature to the specific acclimation temperature began on day 5 at an increase (toward 35 °C) or decrease (toward 25 °C and 20 °C) of approximately 1 °C per day. The 30 °C treatment

Table 1. Overview of temperature acclimation treatments applied to a northern (low latitude) and southern (high latitude) populations of juvenile *Lates calcarifer*.

Population	Control/ intermediate	Cold stress	Heat stress
Northern	30 °C/25 °C	20 °C	35 °C
Southern	25 °C/30 °C	20 °C	35 °C

was considered to be at the required population-specific acclimation temperature on day 1. Once at the required acclimation temperature, all tanks of a given treatment were held at their respective temperatures for 28 days before swimming (U_{crit}) trials. Identical treatment conditions were applied to southern individuals, except that they were initially acclimated to 25 °C for the first 4 days before the 1 °C·day^{-1} change towards the specific acclimation temperatures for each treatment (35 °C, 30 °C, 20 °C). Southern control treatment fish (25 °C) were considered to be at the population-specific acclimation temperature on day 1.

Mortality

A small number of individuals displayed erratic behavior (e.g., loss of equilibrium) synonymous with viral infection (nodavirus) and were immediately removed, euthanized, and excluded from the study. Otherwise, all mortality observed during the experiments was assumed to occur as part of the thermal-induced physiological challenge of the different treatments. For all χ^2 comparisons of mortality (Jonassen et al. 1999), the expected mortality rate for juvenile fish under thermal stress was considered to be 0.36%·day^{-1} for the first 20 days and 0.18%·day^{-1} thereafter (Hokanson et al. 1977).

Swimming speed performance

Following the acclimation period, juvenile *L. calcarifer* were fasted for 36 h prior to U_{crit} trial to ensure a postabsorptive state that maximizes metabolically available energy (reviewed by Fulton 2010; Plaut 2001) and minimizes variations in gut fullness between individuals (Billerbeck et al. 2001). All swimming performance trials were conducted during daylight hours (0530–1930), with water temperature in the flow tank being identical to that of the holding tanks, with the exception of the final experiment (detailed below) that involved swimming fish in three temperatures different to that of their holding tanks. All swimming performance trials were conducted in the 194 L recirculating flow tank described in Fulton (2007).

Each U_{crit} trial began with placing a randomly selected individual from one of the three different holding tanks for each treatment temperature into the working section of the flow tank (55.0 cm length × 11.5 cm width × 24.0 cm height). After an initial still-water acclimation (until resting behaviour was observed), each individual was swum at an acclimation speed of approximately 1.5 total lengths per second (TL·s^{-1}) for 30 min. If no adverse behavior was observed in this acclimation period, the flow speed was increased in increments of approximately 0.33 TL·s^{-1} every 20 min until the trial end point, which was taken as when the fish lost position in the working section and impinged on the downstream grid for more than 5 s. For each individual, the U_{crit} value was calculated using the formula of Brett (1964):

$$U_{crit} = U + [U_i(t \cdot t_i)]$$

where U represents the penultimate speed, t is time swum in final velocity increment, t_i is the time interval in each velocity increment (20 min), and U_i is the velocity increment (2.6, 3.0, 3.3, 3.6, 4.0, and 4.3 cm·s^{-1} for fish with estimated total lengths of 8, 9, 10, 11, 12, and 13 cm, respectively). This U_{crit} procedure was repeated for eight fish from each of the four temperature treatments for both populations (northern and southern).

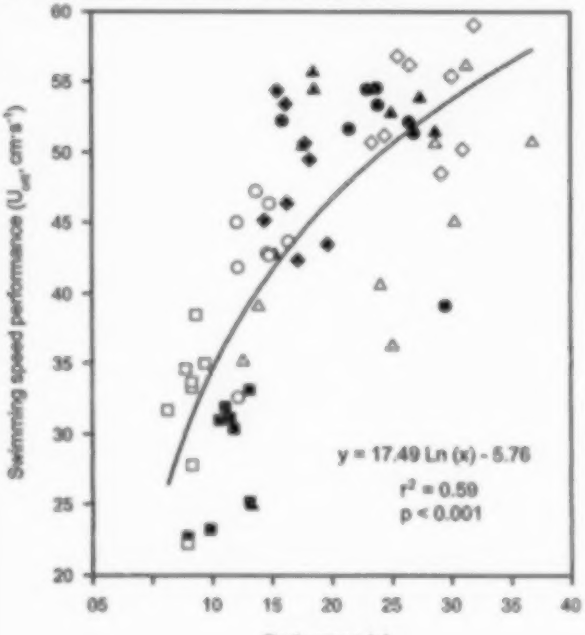
Upon completion of the trial, the test subjects were euthanized with an overdose of clove oil solution (5% clove oil, 15% ethanol, 80% 35 ppt saltwater solution) and then preserved in an ice-water slurry before measuring total length, maximum body depth, maximum body width (to nearest millimetre), and mass (to nearest 0.1 g). These body measurements were used to calculate solid blocking effects following the equations of Bell and Terhune (1970) and Korsmeyer et al. (2002). The average adjustment to U_{crit} for solid blocking effects was a +0.49% increase across the four treatments.

The final set of U_{crit} trials were conducted on a group of 20 °C acclimated southern population fish ($n = 24$). Six individuals were reassessed at 20 °C to confirm that U_{crit} performance had not increased as a result of 7 days of additional acclimation. The remaining fish were swum at 25, 30, and 35 °C without prior acclimation to these temperatures ($n = 6$ per temperature) to measure the phenotypic response of cold-acclimated individuals to an immediate increase in ambient temperature.

Statistical analysis

Mortality rates were assessed for deviation from expected via χ^2 tests following Jonassen et al. (1999). Given positive correlations between body size and speed in the experimental populations (Fig. 1), we statistically controlled for body size in the swimming speed analyses via a general linear mixed model (GLM) analysis with population (northern and southern) and temperature as fixed factors and body size (mass) as a covariate. After running the full model with all terms included, we applied model simplification to hierarchically remove the least significant interaction terms in the model, starting with the higher-order interactions following Crawley (2003). At each step, we performed diagnostics of the residuals to verify that the data were normally distributed with homoscedastic variance. Model simplification was continued until only significant interaction terms and the main effects remained in the model. We then reintroduced each of the nonsignificant interaction terms to calculate their significance level in the final simplified model. Mass-adjusted U_{crit} means were obtained from the final simplified model for visual post hoc comparisons of swimming speed performance with temperature across the two populations. Initial data exploration revealed two major outliers (one northern 25 °C individual and one northern 35 °C individual that were greater than 7 standard deviations from the respective group means) that were removed prior to the above analysis. The additional 20 °C acclimated southern treatment group swum in four different temperatures was

Fig. 1. Overall relationship between mass and critical swimming performance (U_{crit}) by *Lates calcarifer* from two populations acclimated to four different temperatures. Solid (northern, low latitude population) and open (southern, high latitude population) symbols denote the following temperatures: 35 °C (triangles), 30 °C (diamonds), 25 °C (circles), and 20 °C (squares).



examined via one-way analysis of covariance (ANCOVA), with temperature as a fixed factor and body mass as a covariate, following residual diagnostics to confirm normality and homoscedascity of variance. All analyses were performed using SPSS (version 16.0, SPSS Inc., Chicago, Illinois).

Results

Mortality

Mortality rates during the 28-day acclimation were relatively low in temperature treatments considered to be nonstressful, with mortalities of 5.9%, 5.9%, 9.8%, and 17.6% in northern (low latitude) fish and 1.4%, 1.6%, 17.2%, and 53.2% in southern (high latitude) fish in 20, 25, 30, and 35 °C temperature treatments, respectively. Several mortalities from both northern and southern 35 °C treatments (n = 2 and 5, respectively) and 30 °C (n = 1 and 2, respectively) were fish euthanized after viral infection (e.g., nodavirus), which are excluded from the above mortality rates. These virally infected fish were clearly discernable from their erratic behaviour such that they could be excluded from the final swimming trials. Observed mortalities in the northern 20, 25, and 30 °C treatments did not differ significantly from expected (χ^2 test, df = 1, $p > 0.4$), whereas mortality in the northern 35 °C treatment (9 fish) was significantly higher than expected (χ^2 test, df = 1, $p < 0.05$). Conversely, observed mortality of southern fish in the lower temperature treatments (20 and 25 °C) was significantly lower than expected (χ^2 test, df = 1, $p < 0.05$), whereas mortality of southern fish in the higher temperature treatments (11 and 36 fish at 30 and 35 °C, respectively) was significantly higher than expected (χ^2 test, df = 1, $p < 0.02$).

Table 2. General linear mixed model analysis of *Lates calcarifer* swimming performance (U_{crit}) for individuals from two populations (northern and southern).

	F	df	*p*
Simplified model terms			
Constant	160.9	1, 53	<0.01
Population	5.21	1, 53	0.03
Temperature	16.22	3, 53	<0.01
Mass	13.70	1, 53	0.01
Population × temperature	8.30	3, 53	<0.01
Excluded terms			
Temperature × mass	1.08	3, 50	0.37
Population × mass	2.06	1, 52	0.16
Temperature × population × mass	0.86	7, 46	0.55

Note: Animals were grown under four temperatures (20, 25, 30, and 35 ± 1 °C), with body size (mass) as a covariate. Terms of the final simplified model are provided along with nonsignificant interaction terms excluded during model simplification (Crawley 2003).

Critical swimming performance (U_{crit}) and thermal stress

A significant relationship between U_{crit} (in centimetres per second (cm·s^{-1})) and body mass (grams) was observed across all populations and treatment groups (Fig. 1). Simplified GLM analysis revealed significant effects of population, temperature, and mass on U_{crit} in our *L. calcarifer* treatment groups (Table 2). A significant population × temperature interaction term indicated a different response of the northern and southern populations to the temperature treatments. Differences in mass-adjusted U_{crit} within and among populations indicated that the northern population displayed a markedly greater increase in U_{crit} from 20 to 25 °C than the southern population, and the southern population displayed significant variation among 25, 30, and 35 °C treatments, whereas the northern population did not (Fig. 2). Notably, the 20 °C (cold-stress) northern treatment group displayed a significantly slower U_{crit} than the 20 °C southern treatment group (Fig. 2), whereas the 35 °C (heat-stress) northern treatment group significantly outperformed the 35 °C southern treatment group (Fig. 2).

Southern-population fish acclimated to 20 °C for an additional 7 days (35 days total) and then swum across four different temperatures displayed significant differences in U_{crit} with temperature ($F_{[3,23]} = 46.234$, $p < 0.01$) without a significant effect of body mass ($F_{[1,23]} = 2.260$, $p = 0.149$). Such differences were most apparent when these 20 °C acclimated fish were swum at 35 °C (significantly slower), with no significant difference apparent among the other three swimming temperatures (Fig. 3). Although this 20 °C acclimated southern treatment group had an additional 7 days of growth and acclimation, they exhibited a similar U_{crit} (31.77 ± 1.35 cm·s^{-1}, mean ± standard error, SE) to that of the 20 °C southern treatment group acclimated for 28 days (32.10 ± 0.61 cm·s^{-1}). Notably, the trend of reduced

Fig. 2. Mean critical swimming speed performance (U_{crit}) of *Lates calcarifer* from northern (shaded bars) and southern (open bars) populations exposed to four thermal treatments during early growth ($n = 8$ individuals per bar). Note that northern (low latitude) fish exhibit a significantly slower U_{crit} at 20 °C and a relatively greater increase in U_{crit} from 20 °C to 25 °C than southern (high latitude) fish. U_{crit} values are mass-adjusted means ($n = 8$) with 95% confidence limits (CL) calculated from the general linear mixed model (GLM) analysis.

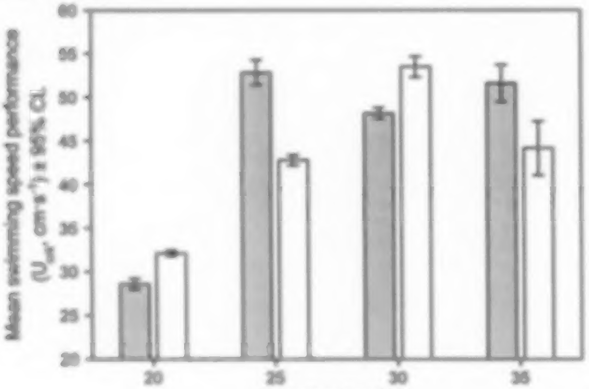
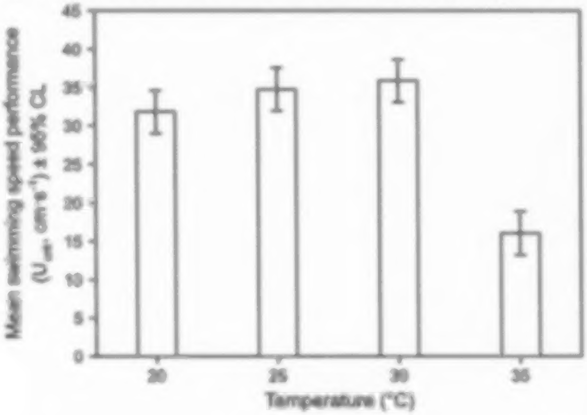


Fig. 3. Mean critical swimming speed performance (U_{crit}) of *Lates calcarifer* from a southern (high latitude) population acclimated to 20 °C during early growth and then swum at four different temperatures without acclimation to those temperatures. U_{crit} values are mass-adjusted means ($n = 6$) with 95% confidence limits (CL) calculated from the general linear mixed model (GLM) analysis.



swimming speed performance at high temperature in this 20 °C group of the southern population is consistent with the above experiment, where speeds were significantly depressed in southern population fish grown under 35 °C (Figs. 2 and 3).

Discussion

Tropical Australian barramundi from geographically and genetically distinct populations display population-specific locomotor phenotypes in response to thermal stress. They do this in a pattern that reflects the thermal environmental history of each population. Specifically, *L. calcarifer* individuals from a southern (high latitude) population displayed higher levels of swimming performance than northern (low latitude) individuals of the same species under cold temperatures (20 °C), while the converse was true at 35 °C. Observed mortalities also indicated strong population-specific responses to thermal stress, with southern fish exhibiting markedly higher mortality at 30 °C and 35 °C than northern fish. Such phenotypic responses, which pivot around the native temperature of each barramundi population, appear to parallel the demonstration that North American *Fundulus heteroclitus* populations perform best (U_{crit}, reported in body lengths per second (BL·s^{-1})) under a treatment temperature most similar to that of their native habitat (DiMichele and Powers 1982). However, it should be noted that a more recent study failed to find similar differences in the swimming performance of wild-caught *F. heteroclitus* populations from the same geographic regions when acclimated over a range of non-native (7 to 33 °C) thermal conditions (Fangue et al. 2008). Of course, there is considerable potential for environmental and genetic change to have occurred in each of these populations in the intervening 26 years between the former and latter studies, which may have influenced the outcomes. Nonetheless, we note that our barramundi populations did have some strong overlap in their levels of swimming performance across an intermediate range of temperatures. Although this confirms the wide thermal tolerance of this species (Katersky and Carter 2005, 2007) and may help explain their extensive distributional range in Australia at extreme shifts in temperature above and below native conditions, we did find significant reductions in swimming speed performance and survival that were largely consistent with our expectations. Given the central importance of swimming in the biology and ecology of fishes, such reductions in locomotor capacity at thermal extremes may have serious implications for patterns of growth, reproduction, and survival in this species (Blake 2004).

Population-specific changes in the magnitude and direction of locomotor performance and mortality appear to mirror the environmental history of the two barramundi populations in this study. Seasonal mean temperatures typically differ by 5–10 °C between the tropical and subtropical native habitats of the two *L. calcarifer* populations examined here, with minima often reaching 20 °C or below in the southern region and mostly above 25 °C in the north (Georges 1992; Pusey et al. 2004; Rea et al. 2002). Combined with the fact that these populations are both geographically and genetically distinct (the latter arising because of the nonoverlapping nature of these breeding populations; Chenoweth et al. 1998; Keenan 1994), there is a strong potential for population-level locomotor phenotypes to be linked to unique allozyme and microsatellite signatures within each of these populations (Chenoweth et al. 1998; Keenan 1994). Though such a genotypic link is yet to be determined in *L. calcarifer*, variations in critical swimming performance in these ectothermic species may be closely linked to the ability to maintain aerobic metabolic processes such as an ability to process muscle lactate across a range of temperatures (Claireaux et al. 2006; Fangue and Bennett 2003; Jain and Farrell 2003). Such a population-specific molecular response has been detected for the lactate dehydrogenase-B locus transcript (*ldh-b*) and encoded lactate-processing enzyme LDH-B (i.e., converts lactate back to pyruvate or glucose via oxidation or gluconeogenesis, respectively) in genetically distinct populations of North American *F. heteroclitus* (reviewed by Powers and Schulte 1998; Schulte 2001). A similar metabolic mechanism may provide further explanation for the phenotypic differences seen in the locomotor phenotypes of our barramundi populations.

Notably, the U_{crit} trials conducted on 20 °C acclimated southern individuals across a range of temperatures both confirmed the above trends and revealed some surprises in the phenotypic response of a cold-water population of barramundi. Essentially, these experiments provided an indication of how cold-water individuals would respond to an instantaneous change in temperature. Following our hypothesis, locomotor performance was substantially depressed at the highest nonacclimation temperature (35 °C). However, rather than displaying the highest speed at their acclimated temperature (20 °C), these cold-water individuals displayed remarkably similar (high) swimming speeds across a broad range of nonacclimation temperatures (20–30 °C). Such a breadth of thermal tolerance in this group may be a reflection of the rapid changes in water temperature that these fish can experience throughout a diel cycle in a tropical Australian river (as much as 15 °C per day; Georges 1992; Pusey et al. 2004; Rea et al. 2002).

In conclusion, we find that *L. calcarifer* from genetically distinct populations exhibit markedly different phenotypic responses to alterations in their thermal environment. The fact that each population exhibits higher swimming speed performance following acclimation to their respective native environments (35 °C and 20 °C for northern and southern populations, respectively) suggests an intrinsic adaptation of each population to the thermal regime to which they are evolutionarily accustomed. Of value in future research would be an attempt to uncover the underlying physiological and genotypic mechanism(s) that may help explain such phenotypic responses of different fish populations to thermal stress. Given the aerobic nature of swimming performance, a key area in which to begin may be an examination of thermally sensitive enzymatic activity using carefully selected molecular tools (e.g., candidate genes) to begin to unravel the genotypic basis for phenotypic differences (e.g., U_{crit}) in discrete wild populations.

Acknowledgements

The authors thank Russell Graham and Norton Perry for aquarium assistance and Dean Jerry (James Cook University) and Bill Foley (The Australian National University) for storage and transportation of samples. The authors also thank Simon Fisher (Queensland Department of Environment and Resources Management) for river flow and temperature data. Funding was provided by the Research Advancement Program in Finfish Aquaculture Grant (L.v.H.) and the Australian National University (C.J.F.). Experimental methods were performed under approval from the Australian National University Animal Experimentation Ethics Committee (No. F.BTZ.03.06) and the James Cook University Animal Ethics Committee (A1118). This research was conducted in Ngunnawal Country.

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Estimating bioenergetics model parameters for fish with incomplete recapture histories

Bradley E. Thompson and Daniel B. Hayes

Abstract: Traditional methods for modeling growth of free-ranging fish are often limited by missing recapture observations that prevent individual growth estimates for a given time interval. Our purpose is to present a method for modeling growth rates of juvenile steelhead (*Oncorhynchus mykiss*) that addresses this limitation. Age-1 juvenile steelhead were individually marked with passive integrated transponder (PIT) tags, released in a Michigan, USA, watershed, and sampled monthly (May–November) with barge electrofishing. Individual growth was modeled using daily water temperature and observed fish sizes as inputs and by determining the proportion of maximum consumption parameter (*P*) for the bioenergetics equation that provided a minimum residual squared error. Results demonstrate that individual steelhead growth can be accurately modeled using water temperature and a temporally specific *P* shared by all individuals. Advantages of using this method to model fish growth include the ability to bridge data gaps where observations are lacking in individual length histories, rigorously test for differences in *P* across time periods, and estimate variability of *P* among fish within a given stream reach.

Résumé : Les méthodes traditionnelles de modélisation de la croissance des poissons libres en nature sont souvent restreintes par le manque d'observations de recaptures qui empêche l'estimation des croissances individuelles pendant un certain intervalle de temps. Notre objectif est de présenter une méthode de modélisation des taux de croissance de jeunes truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) qui résoud ce problème. Nous avons marqué individuellement des jeunes truites arc-en-ciel anadromes d'âge 1 d'une étiquette PIT (transpondeur intégré passif), les avons libérées dans un bassin versant du Michigan, É.-U., et les avons échantillonnés chaque mois (mai–novembre) par pêche électrique sur barge. Nous avons modélisé la croissance individuelle en utilisant les températures journalières de l'eau et les tailles de poissons observées comme données d'entrée et en déterminant la proportion du paramètre de consommation maximale (*P*) de l'équation bioénergétique qui produit l'erreur au carré résiduelle minimale. Nos résultats démontrent qu'il est possible de modéliser avec précision la croissance individuelle des truites arc-en-ciel anadromes à partir des températures de l'eau et d'une valeur de *P* spécifique au temps et commune à tous les individus. Les avantages d'utiliser cette méthode pour la modélisation de la croissance des poissons incluent la capacité de combler des lacunes dans les données lorsqu'il manque des informations sur l'évolution des croissances individuelles, de tester rigoureusement les différences de *P* entre les diverses périodes et d'estimer la variabilité de *P* entre les poissons d'une même section de cours d'eau.

[Traduit par la Rédaction]

Introduction

Bioenergetics models are a popular tool for providing insight into the growth dynamics of fishes. These models have numerous applications in fisheries science, with two common themes being (*i*) how do environmental conditions influence the growth of fish and (*ii*) what are predicted levels of prey consumption given observed growth of fish in a population (Hartman and Kitchell 2008). Applications of these models to fish in the wild typically involves fixing most model parameters at values obtained from laboratory experiments or the literature and then allowing a subset of parameters to vary to obtain a "good" fit between the observed and predicted growth (e.g., Hartman and Kitchell 2008). This is commonly done at the population level, where the mean size at age is used to represent growth (e.g., Sweka and Hartman 2008).

Technological advancements (e.g., passive integrated transponder (PIT) tags) provide new and less expensive ways of marking individual fish, increasing the amount of information available for bioenergetics modeling of fish in the wild. Individually identifiable tags provide many advantages for understanding the growth dynamics of free-ranging fishes in the wild. Most important to this study, tagging ensures that information from the same fish observed during

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Received 17 June 2009. Accepted 5 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 11 June 2010.
J21262

Paper handled by Associate Editor Kenneth Rose.

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doi:10.1139/F10-048

subsequent sampling events through time can be tracked. Secondly, tagging combined with frequent sampling allows for the estimation of temporal variation in growth of individuals within a given site because it provides an opportunity to compute an individual's growth rate between individual observations. As noted by Hartman and Kitchell (2008), there is an increasing trend of incorporating bioenergetics considerations in individual-based models, leading to a greater need for field estimates of individual variation.

Despite the advantages of using individually tagged fish, an unresolved problem is that field sampling rarely allows for the capture of all individuals at large. The result is a data set that can be viewed as a matrix of sampled and missing size observations (Fig. 1). Missing recapture observations prevent direct calculation of individual growth for all fish during each time interval. As an example, the second fish in Fig. 1 was not observed in the third sampling event. Therefore, growth for this individual cannot be directly calculated for each of the second and third time intervals. At best, growth can be calculated for the combined period spanning the second and third time intervals. However, if environmental conditions, particularly food resources, vary between time intervals and we are interested in how these changes affect growth, then the combined growth calculation is not ideal.

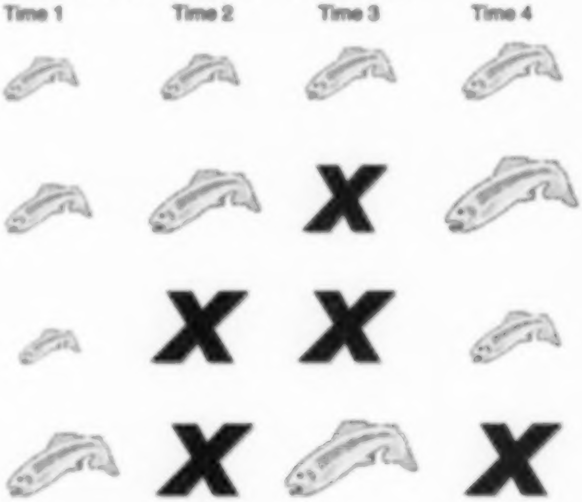
The primary goal of this paper is to present a statistical approach for modeling growth of free-ranging, individually tagged fish not observed on all sampling events. More specifically, we demonstrate how size data from other tagged individual fish in the same stream reach, stream temperature data, and a bioenergetics model can be used to estimate the proportion of maximum consumption parameter for fish during time with missing size observations. Our approach is based on the statistical principle of nonlinear least squares fitting and allows for the calculation of standard errors of parameter estimates, which, to our knowledge, has not been done before. In addition to our main goal, the approach we develop allows for the determination of fish-specific parameter estimates, useful for quantifying individual variation. We illustrate how results of this method provide useful inputs to individual-based models of fish growth. Finally, we apply this technique to tagging and recapture data on age-1 steelhead (*Onchorynchus mykiss*) in a Great Lakes tributary, showing how proportion of maximum consumption rate estimates vary spatially, temporally, and among individuals.

Materials and methods

Study area

Pine River, a tributary to the Au Sable River and Lake Huron, drains a 756 km^2 watershed located in the southeast quarter of Alcona County, Michigan, USA. Pine River is a typical Michigan coldwater trout stream, generally maintaining a daily mean water temperature <22 °C during the warmest summer months (Fig. 2). The fish community is dominated by juvenile steelhead, brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), mottled sculpin (*Cottus bairdii*), creek chub (*Semotilus atromaculatus*), white sucker (*Catostomus commersoni*), and northern brook lamprey (*Ichthyomyzon fossor*).

Fig. 1. Hypothetical fish sampling outcome in which one individual (top row) is observed on all four sampling events and three individuals (lower three rows) were not observed on at least one sampling date. A fish symbol indicates that a size measurement was recorded; an "X" indicates no size observation.



Tagging of steelhead

Wild age-1 steelhead (80–180 mm) were collected at 11 sites (Fig. 3) in the Pine River watershed using tow-barge electrofishing during May and June of 2001 and 2002. Fish were individually marked with a 12 mm 125 kHz passive integrated transponder (PIT) tag inserted into the abdominal body cavity 5 mm anterior of the pelvic fins with a 12 gauge needle and implanter syringe. The adipose fin was also clipped to aid in identification of tagged fish during recapture events. We recorded the unique tag number and total length to the nearest millimetre for each fish and transferred the fish to an aerated water cooler to recover for 10–15 min. The above procedure was repeated for batches generally ranging from 30 to 100 fish, and fish were then released at our study sites. Total number marked at each site each year ranged from 49 to 165 fish; total number of recapture events in sites included in our analysis (i.e., including multiple recaptures of individuals) ranged from 19 to 138 observations.

Recapture sampling

In both years, study reaches were sampled approximately monthly from June through early November using tow-barge electrofishing to recapture tagged individuals. Because fish were observed to disperse widely, sampling often covered 1000 m or more, and two upstream passes were conducted to increase recapture numbers. Within a given pass, steelhead captured were kept in an aerated water cooler. After each pass, these fish were transferred to an aerated water cooler located on the stream bank at the midpoint of the study reach. All fish collected from both passes were measured to the nearest millimetre for total length, scanned with the portable reader to record tag number, and returned to the stream. Although weight can be measured relatively quickly, our sampling involved electrofishing river reaches in excess of 1 km per day. Further, our sampling was for multiple pur-

Fig. 2. Mean daily water temperature of the South Branch of Pine River during May to November 2001 (broken line) and 2002 (continuous line).

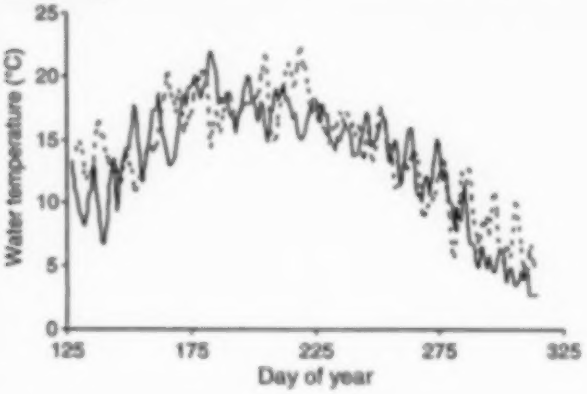
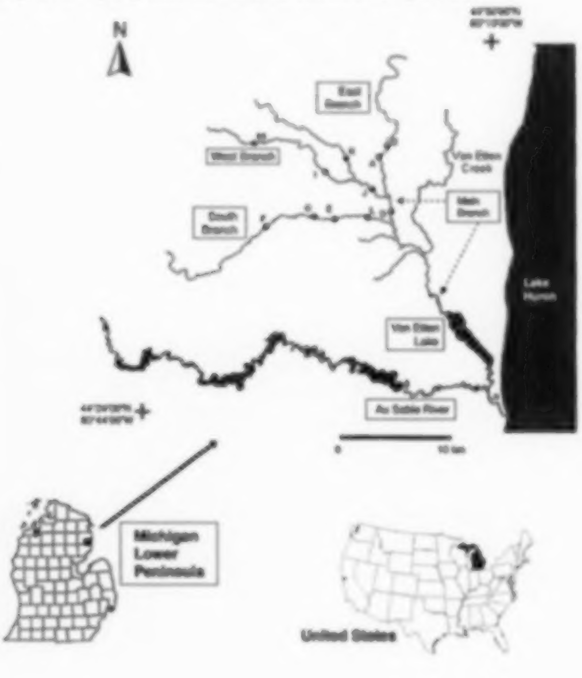


Fig. 3. Sampling sites within Pine River, Michigan, USA.



poses, and as such, there was insufficient time to collect fish weights in addition to length. Because we measured fish size as total length and measures of weight are needed for bioenergetics modeling, we converted length (in millimetres) to weight (in grams) using the standard length–weight equation:

$$\log_{10}(\text{weight}) = -5.023 + 3.024 \cdot \log_{10}(\text{length}) \tag{1}$$

for lotic rainbow trout (Anderson and Neumann 1996).

Table 1. Juvenile steelhead (*Oncorhynchus mykiss*) consumption, respiration, egestion, and excretion parameters used in the bioenergetics model, following Hanson et al. (1997).

Equation and parameter	Value
Consumption eq. 3	
CA	0.628
CB	–0.3
CK1	0.2
CK4	0.2
CQ	3.5
CTL	24.3
CTM	22.5
CTO	25
Respiration eq. 2	
RA	0.013
RB	–0.217
RTM	26
RTO	22
RQ	2.2
Egestion	
PFF	0.1
FA	0.212
FB	–0.222
FG	0.631
Excretion	
UA	0.0314
UB	0.58
UG	–0.299
SDA	0.172

Water temperature monitoring

One optic Stowaway® temperature recorder was deployed in the middle of each study reach. Water temperature was recorded every hour from initial tagging until the last recapture event. To minimize loss of Stowaways, each recorder was attached to the top of a 1.2 m long, 9.53 mm diameter steel rod hammered vertically into the streambed with all but 0.15 to 0.33 m of the rod buried in the streambed.

Model description and structure

We modeled the growth of tagged steelhead using an individual-based bioenergetics model, based on the Wisconsin model (Hanson et al. 1997), that we coded in Microsoft Excel. Broadly speaking, the Wisconsin model uses fish weight (grams), water temperature, and a number of other parameters to predict change in weight over time. As in many previous studies (for recent review, see Hartman and Kitchell 2008), we fixed most model parameter values (Table 1) consistent with Railsback and Rose (1999) and allowed the proportion of maximum consumption parameter (P) to vary to best match predicted and observed weights at age. Thus, weight (W) for an individual fish (j) at a time in the future ($t + 1$) is a function of the weight at the present time ($W_{t,j}$), temperature (Temp$_j$), and the proportion of maximum consumption:

$$W_{t+1,j} = W_{t,j} + f(\text{Temp}_t, P_{t,j}) \tag{2}$$

Table 2. Structure of the individual-based juvenile steelhead (*Oncorhynchus mykiss*) bioenergetics growth model.

Day (t)	Water temperature (Temp_t)	Observed weight (W_t)	Predicted weight ($\widehat{W}_t$)	Proportion maximum consumption (P_t)	Growth (G_t)
127	Temp_{127}	W_{127}	W_{127}	$P_{127} = P_1$	G_{127}
128	Temp_{128}		$\widehat{W}_{127} + G_{127}$	$P_{128} = P_1$	G_{128}
129	Temp_{129}		$\widehat{W}_{128} + G_{128}$	$P_{129} = P_1$	G_{129}
	Temp_t		$\widehat{W}_{t-1} + G_{t-1}$	$P_t = P_1$	G_t
156	Temp_{156}		$\widehat{W}_{155} + G_{155}$	$P_{156} = P_1$	G_{156}
157	Temp_{157}	W_{157}	$\widehat{W}_{156} + G_{156}$	$P_{157} = P_2$	G_{157}
158	Temp_{158}		$\widehat{W}_{157} + G_{157}$	$P_{158} = P_2$	G_{158}
159	Temp_{159}		$\widehat{W}_{158} + G_{158}$	$P_{159} = P_2$	G_{159}
	Temp_t		$\widehat{W}_{t-1} + G_{t-1}$	$P_t = P_2$	G_t
311	Temp_{311}	W_{311}	$\widehat{W}_{310} + G_{310}$	$P_{311} = P_2$	G_{311}

Note: Proportion maximum consumption (P_t) was estimated for intervals spanning fish sampling events. Daily growth (G_t) was calculated as a function of consumption, egestion, excretion, respiration, swimming speed, and predicted weight from the previous day ($\widehat{W}_{t-1}$) according to formulas in Hanson et al. (1997).

Each model was run on a daily time step using mean daily water temperature measured by the Stowaway. Similar to previously published steelhead and rainbow trout (*Oncorhynchus mykiss*) bioenergetics models (Rand et al. 1993; Railsback and Rose 1999), we formulated our model using consumption eq. 3 and respiration eq. 2 Wisconsin model options. Proportion digestible and indigestible prey were set to fixed values of 0.90 and 0.10, respectively, similar to the Rand et al. (1993) steelhead model. Also similar to Railsback and Rose (1999), we used an energy content value that was constant for all time periods and individuals of 5900 J·g wet weight^{-1} for trout and 2500 J·g wet weight^{-1} for their prey.

With individually tagged fish, it would theoretically be possible to calculate a $P_{t,j}$ for each individual for each time interval. However, not all individuals are caught on every sampling event. Therefore a $P_{t,j}$ cannot strictly be calculated for all time intervals for all individuals. To address this problem, we assigned a single P to all fish for a given time interval (as illustrated in Table 2). According to this structure, $P_{t,j}$ is simplified to a time-specific value that is common to all fish:

$$(3) \qquad P_{t,j} = P_t$$

This structural detail of the model is critical because it allows for estimation of a $P_{t,j}$ common to all individuals, even if some fish have missing observations either at the start or end of the time interval. Thus, this approach relies on the principle of "borrowing strength" (Pfeffermann 2002) whereby data on individuals that are observed in consecutive sampling events are used to bridge gaps in observations for individuals with missing observations. Using this approach, the fish's initial weight is treated as a fixed starting value, and weight over time is predicted from the model based on this initial weight. In a later section (Quantifying individual variability in P), we describe how individual variation in P can still be incorporated within this modeling structure.

Estimation procedure for *P*

We used the Microsoft Excel Solver Tool to find the set of P_t parameter values that minimized the residual sum of squared deviations (RSS) between observed size and predicted size ($\widehat{w}_t$) simultaneously for all P_t and all fish according to the following equation:

$$(4) \qquad \mathrm{RSS} = \sum_{j=1}^{n} (w_{t,j} - \widehat{w}_{t,j})^2$$

As indicated above, the weight of each fish at first capture was used as a starting value and thus was not used in computing RSS. For each model, observed sizes from all fish recaptured at least once were used to optimize P_t. For each minimization, the following Solver default options were selected: precision = 0.000001, tolerance = 5%, convergence = 0.0001, tangent estimates, central derivatives, and Newton search.

The standard error of estimates of P_t was calculated using a likelihood approach (Ratkowsky 1983). In this approach, the sums of squared errors were first converted to a concentrated likelihood (Seber and Wild 1989), assuming normality of error terms. The first and second derivatives of the concentrated likelihood were then determined from the response of the concentrated likelihood to a 1% change in parameter values from the optimum. We used these derivative estimates to calculate the information matrix and then inverted the information matrix to obtain the variance–covariance matrix of the parameter estimates (Ratkowsky 1983). For details on formulae, we refer the reader to Workman et al. (2002).

Model selection procedure

For a given experiment (year–site combination), several models were constructed in a nested design with models varying only in the number of P_t parameters fitted. The first model constructed for each experiment contained a P_t parameter for each recapture event and thus was the most parameter-rich model, which we refer to as the full model. Subsequent models, with one less P_t parameter than the previous model, were constructed by aggregating P_t for adjacent time intervals with the least difference in estimated P_t values. We refer to these as reduced models. Continual reduction eventually led to a final P_t model, with a single P_t in the model. It is expected that the most parameter rich model in such a set of nested models should have the least model error (RSS). However, in the interest of selecting a parsimonious model for each experiment, we compared models varying in only the number of P_t parameters by using an *F*-test procedure where the *F* statistic is

$$F = \frac{\mathrm{RSS}_{(i)} - \mathrm{RSS}_{(i+1)}}{df_{(i)} - df_{(i+1)}} / \sigma^2_{(i+1)} \tag{5}$$

and where

$$\sigma^2_{(i+1)} = \mathrm{RSS}_{(i+1)}/\mathrm{df}_{(i+1)}$$

$$\mathrm{df}_{(i+1)} = n - p_{(i+1)}$$

$$\mathrm{df}_{(i)} = n - p_{(i)}$$

and *i* is the number of P_t parameters in the reduced model to select the "best" model for each site (Quinn and Deriso 1999). As a conservative approach, the number of fish used in each model was defined as the effective sample size (*n*) used to calculate *F* statistics. Numerator and denominator degrees of freedom for table values were defined as $\mathrm{df}_{(i+1)} - \mathrm{df}_{(i)}$ and $\mathrm{df}_{(i+1)}$, respectively. A separate *F* test was performed for all combinations of models and was compared with critical values ($\alpha = 0.05$) of the *F* distributions. We considered using Akaike information criterion (AIC) as a basis for model selection; however, we chose to use an *F*-test approach because (*i*) the *F* test is appropriate to the nested structure of our model and (*ii*) our intent was to conduct tests of significance between models of varying complexity (e.g., Neter et al. 1993; Burnham and Anderson 2002).

Quantifying individual variability in *P*

In the above models, the same P_t value applied to all fish for a given time interval. To represent variability of *P* among individuals within an experiment, we extend the model to include a fish-specific random effect. This was accomplished by incorporating an additional parameter (r_j) that served as a multiplier for the P_t for each fish, thereby representing individual variation in *P*. We refer to this model as the multiplier model. In this model, $P_{t,j}$ can thus be expressed as

$$P_{t,j} = r_j \cdot P_t \tag{6}$$

where r_j is the multiplier specific to fish *j*. It is important to emphasize that in this structure, r_j varies among individual fish but remains constant over all time intervals for each fish.

A single multiplier model was constructed for each experiment using the model structure (i.e., number of P_t parameters) identified in the *F*-test procedure as being the most parsimonious. The Microsoft Solver tool was used to simultaneously optimize all P_t and r_j by minimizing the sum of squared deviations between observed size and predicted size for all fish. We eliminated potential confounding from simultaneous optimization of P_t and r_j by adding the constraint of maintaining the average of all $r_j = 1.0$. After optimization, we applied the Kolmogorov–Smirnov test of normality and calculated variance to describe the distribution of multiplier factors within each experiment. We used the results of an optimized multiplier model, including the distribution of r_j, in a projection of growth to illustrate a potential application of these parameters.

Table 3. Tagging and recapture frequency of PIT-tagged age-1 steelhead (*Oncorhynchus mykiss*) in the South Branch of Pine River, 2001–2002.

		No. of tagged fish	
Year	Day of year	Tagged	Recaptured
2001	127	52	
	157		17
	184	22	
	190		7
	214		18
	215		3
	273		8
	314		7
2002	127	50	
	140	50	
	161		0
	161	33	
	168	32	
	191		13
	197		9
	210		9
	225		9
	269		6
	311		3

Growth model construction and performance

We illustrate the details of our modeling approach by applying the model to one site (South Branch site F) sampled in 2001 and 2002. Our initial presentation highlights the model selection process used to develop a parsimonious model. A multiplier model, which included fish-specific r_j, is presented for this site for 2001 and 2002 to show how individual variation can be incorporated. To illustrate the use of the multiplier model for projecting growth of age-1 steelhead, we simulated the range of growth trajectories for steelhead starting at a length of 100 mm (10.6 g) for day 127 through day 311.

The multiplier model was applied to each of 13 tagging experiments that we conducted in 2001 and 2002, spread across the Pine River watershed (Fig. 3). Results of these models were used to determine the variability in estimates of *P* among sites in the watershed, as well as across years.

Results

Growth model construction and performance

A total of 74 and 165 age-1 steelhead were captured, PIT-tagged, and released at South Branch site F during May and June of 2001 and 2002, respectively (Table 3). The total number of individuals recaptured at least once was 33 in 2001 and 36 in 2002. The number of steelhead recaptured per sampling event ranged between 3 and 18 for six recapture samples in 2001 and between 0 and 13 for eight samples in 2002 (Table 3). In both years, the number of recaptured fish generally decreased during the progression

Table 4. Optimized P_t values (initial day – final day of time interval), residual sum of squared deviations (RSS), and test of significant model improvement (F) between models are shown for each of the six nested models optimized for the South Branch of Pine River in 2001 and 2001.

(*a*) Year 2001.

No. of P_t parameters in the model	P_1 (127–156)	P_2 (157–183)	P_3 (184–189)	P_4 (190–214)	P_5 (215–272)	P_6 (273–314)	RSS	F
6	0.174	0.076	0.116	0.116	0.071	0.120	976.66	
								2.05×10^{-6}
5	0.174	0.076	0.116		0.071	0.120	976.66	
								2.197
4	0.174	0.078	0.112		0.081		1053.28	
								1.139
3	0.162	0.102			0.084		1094.64	
								1.376
2	0.173	0.091					1144.85	
								26.011*
1	0.102						2105.44	

(*b*) Year 2002.

No. of P_t parameters in the model	P_1 (127–160)	P_2 (161–190)	P_3 (191–209)	P_4 (210–224)	P_5 (225–268)	P_6 (269–311)	RSS	F
6	0.316	0.109	0.125	0.070	0.162	0.166	3297.90	
								0.002
5	0.316	0.109	0.125	0.067	0.163		3298.12	
								0.041
4	0.314	0.114		0.076	0.163		3302.50	
								0.225
3	0.318	0.108			0.158		3325.74	
								3.052
2	0.272	0.132					3633.30	
								8.616*
1	0.155						4554.00	

Note: A total of 33 and 36 fish (n for each model) were used in each of the 2001 and 2002 models, respectively, to calculate F. An asterisk (*) indicates significance at $\alpha = 0.05$.

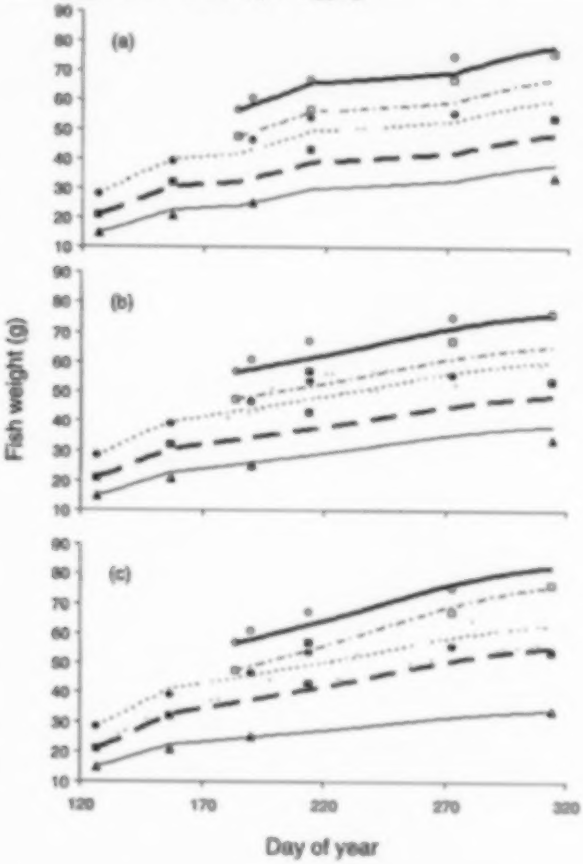
of the study. No fish tagged in 2001 were recaptured during 2002.

In both years, the most parameter-rich model (full model) contained six P_t estimates, and the most reduced model contained a single P_t for the entire study period (Table 4). For the most parameter-rich model, interval length ranged between 5 and 57 days during 2001 and between 18 and 43 days during 2002 (Table 4). Model error (RSS) increased with decreasing model complexity (Table 4). In both years, reductions in the number of P_t parameters from 6 to 2 resulted in small increments of model error (Table 4) that were not statistically significant. In contrast, a significant reduction in model error (2001, $F = 26.011$, df = 1, 32; 2002, $F = 8.616$, df = 1, 35) occurred between models containing one and two P_t, indicating that a two-parameter model provided the best fit while maintaining parsimony (Table 4). In both years, estimates of P_t for the first time period were substantially higher than for the second time period (Table 4).

Model performance for both the most parameter-rich and the F-selected model was relatively good for PIT-tagged juvenile steelhead in both years. Results for the most parameter-rich model for 2001 and 2002, respectively, are illustrated (Figs. 4*a* and 5*a*). All fish show parallel growth trajectories that vary within the year given six time intervals when P_t differed. As is evident for these fish, predicted weights followed observed weights quite closely, with no evidence of temporal bias. Results for the two-parameter model that was selected based the F-test results for each year are also illustrated (Figs. 4*b* and 5*b*). Growth trajectories were parallel, showing rapid early growth (days 127–156 in 2001 and days 127–160 in 2002) and steady but lower growth for the remainder of the sampling season. Although these models had four fewer P_t parameters, model error was not significantly greater. In both years, the accuracy of predicted size was similar for fish tagged during May (day 127) or at a later date (Figs. 4 and 5).

In 2001 and 2002, patterns in the estimates of the two P_t parameters for the multiplier model for the South Branch site were similar to P_t parameter estimates of the F-selected model in each year (Tables 4 and 5). The difference in slope of the predicted weights (Figs. 4*c* and 5*c*) illustrates the extent of growth rate variation among individuals. In addition to allowing growth rate to vary among individuals, the inclusion of r_j greatly improved model performance, as indicated by a decrease in model RSS from 1144.85 to 155.86 in 2001 and from 3633.30 to 146.15 in 2002 (Tables 4 and 5). In both years, the distribution of r_j was not significantly different from a normal distribution (2001, Kolmogorov–

Fig. 4. Comparison of observed and predicted sizes using the (*a*) P_0, (*b*) *F*-selected P_2, and (*c*) multiplier P_2 models for five free-ranging passive integrated transponder (PIT) tagged age-1 steelhead (*Oncorhynchus mykiss*) in the South Branch in 2001. In this example, five fish with four or more recaptures were selected to illustrate model fit. All predicted weights (lines) start with the observed size of a given fish on the day of tagging.



Smirnov $D = 0.073$, $p > 0.15$; 2002, Kolmogorov–Smirnov $D = 0.0946$, $p > 0.15$; Fig. 6). Structurally, the mean of r_j was 1.0, and the standard deviation of r_j was 0.187 in 2001 and 0.219 in 2002.

The consequence of the differences in both P_t and water temperature between 2001 and 2002 at the South Branch site was a faster growth rate and larger size for fish in 2002 relative to 2001 (Fig. 7). The higher variation in r_j in 2002 also led to a broader interquartile range in 2002 than in 2001 (Fig. 7).

Fig. 5. Comparison of observed and predicted sizes using the (*a*) P_0, (*b*) *F*-selected P_2, and (*c*) multiplier P_2 models for five free-ranging passive integrated transponder (PIT) tagged age-1 steelhead (*Oncorhynchus mykiss*) in the South Branch in 2002. In this example, five fish with four or more recaptures were selected to illustrate model fit. All predicted weights (lines) start with the observed size of a given fish on the day of tagging.

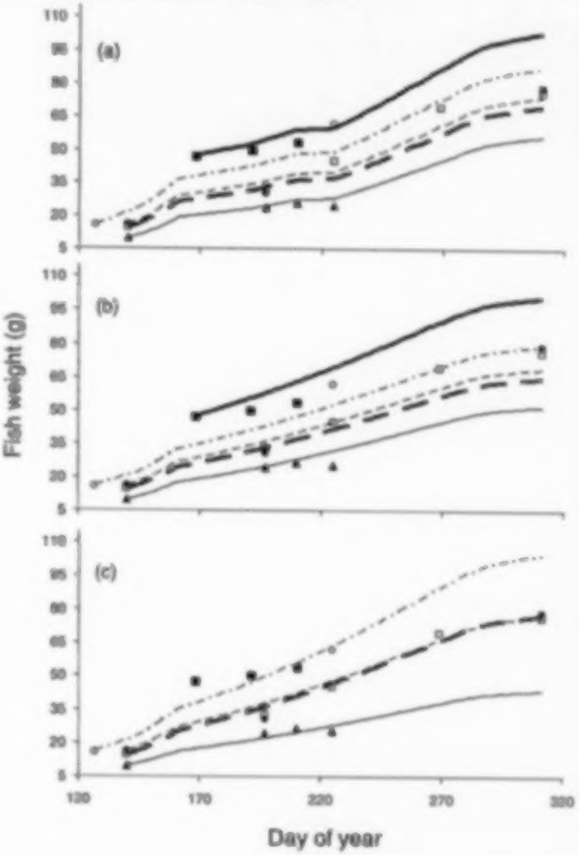


Table 5. Optimized P_t values (±SE) and residual sum of squared deviations (RSS) for each P_t multiplier model (2001 and 2002 South Branch campground), which included individual fish r_j.

Model	P_1	P_2	RSS
2001	0.168±0.004 (days 127–156)	0.083±0.001 (days 157–314)	155.86
2002	0.230±0.003 (days 127–160)	0.119±0.001 (days 161–311)	146.15

Note: RSS calculated as the sum of squared differences between all predicted and observed fish sizes.

Application to age-1 steelhead in a Great Lakes watershed

A two-parameter model was most parsimonious in 12 of the 13 tagging experiments, and a one-parameter model was selected for one experiment (Table 6). As with the South Branch experiments presented in detail above, the first P_t in most experiments generally represented consumption during May, with the second P_t representing June through the end of the growth season (Thompson 2004). Across our 12 experiments with two time intervals, P_t was more than double for May (mean = 0.218, SE = 0.020) than for the remainder of the growing season (mean = 0.102, SE = 0.007). Although some site-to-site variation was evident, the differences among sites were much less than between years

Fig. 6. Frequency histogram of r_j multipliers from optimized multiplier models for the South Branch in (*a*) 2001 and (*b*) 2002. Values on the *x* axis are centered on bin midpoints.

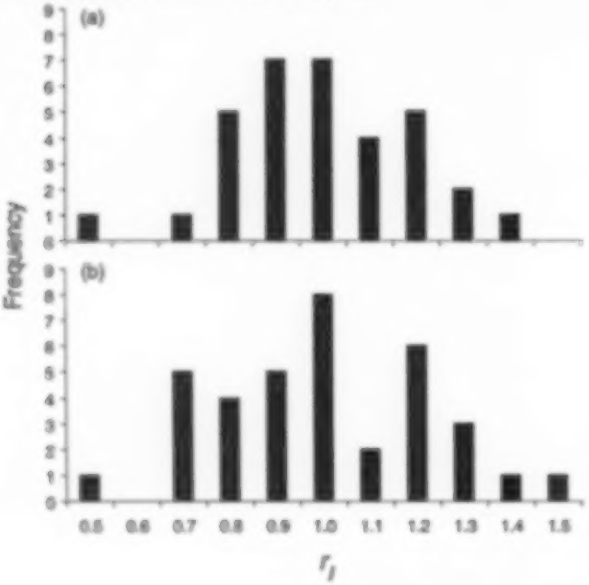
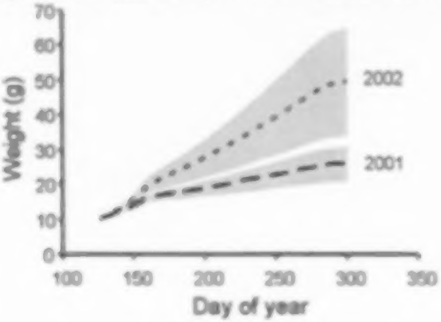


Fig. 7. Predicted mean weight and interquartile range of age-1 steelhead (*Oncorhynchus mykiss*) (starting at 10 g on day 127) comparing 2001 and 2002 in the South Branch of Pine River.



(Table 6), with estimates of *P* consistently lower in 2001 than 2002 (Table 6). The standard deviation of the multipliers (r_j) did not vary between years (t = 1.24, $p > 0.05$) and averaged 0.201 for both years combined. The distribution of the r_j was not significantly different than normal in 11 of 13 experiments (Table 6).

Discussion

Results from our 13 experiments spread across multiple sites in two years revealed interesting biological insight into age-1 steelhead growth. After accounting for the effects of variation in water temperature in the overall bioenergetics model, estimates of the proportion of maximum consumption (*P*) were consistently much higher in May than during the remainder of the year, emphasizing the importance of spring feeding for stream-resident steelhead. Similar patterns of higher growth of juvenile steelhead during spring when water temperatures are warming rapidly were observed by Hayes et al. (2008) in a coastal California stream. Our observation of higher *P* is also consistent with seasonal patterns of diet and stomach fullness of brown trout and rainbow trout in another Michigan stream (Mistak et al. 2003), as well as patterns of stream insect drift reported in the literature (e.g., Wipfli 1997). Estimates of *P* for young steelhead reported in the literature were generally near 0.30 (Railsback and Rose 1999; Godby et al. 2007; Paukert and Petersen 2007). Our estimates of *P* were substantially lower than this value, particularly for midsummer periods. Part of the reason for this difference may be differences in other parameters within the bioenergetics model, differences in the size range of fish or water temperature (which affect $C_{\max}$ and *P*), and differences in growth rates at each site. For example, growth rates in the Godby et al. (2007) study were between 1.2% and 1.9% per day, whereas our growth rates were often less than 1% per day.

We observed larger differences in *P* among years than among sites within a year, indicating that factors affecting food production varied more among years than sites. Moreover, these differences were evident even after the impact of annual variation in water temperature was considered. Results of simulations in which fish were "virtually transplanted" (e.g., Garvey and Marschall 2003) indicate that the impact of annual variation in *P* on overall growth was much larger than the impact of variation in water temperature among sites and years (Thompson 2004). Therefore, it appears to be important to sample across several years to adequately represent growth within watersheds similar to the Pine River watershed.

These results illustrate how a bioenergetics model constructed with inputs of initial fish size, water temperature, and time-varying *P* can bridge gaps in individual length histories and accurately model growth of free-ranging steelhead. In this paper, we address several substantial issues raised in recent overview papers published on bioenergetics models (Chipps and Wahl 2008; Hartman and Kitchell 2008). In particular, Chipps and Wahl (2008) highlight the need for hypothesis testing within a bioenergetics framework, a need that can be addressed with the formal statistical tests that we illustrate in this paper. These methods can be used to develop parsimonious models of intra-annual variation in consumption, as well as approximate standard errors for parameter estimates. Hartman and Kitchell (2008) highlight the increasing dependence of individual-based models on bioenergetics models. We demonstrate how inclusion of a fish-specific random effect (r_j) provides a means of estimating and representing variation among individuals, providing a direct way of connecting these modeling approaches.

However, application of these methods raises important questions that should be considered when using bioenergetics models. A fundamental issue with bioenergetics model application is the accuracy with which important ecological processes are represented. For example, as summarized by Chipps and Wahl (2008), over 80% of estimates of consumption in free-ranging fishes from bioenergetics models were greater than estimates obtained directly from diet samples. Because of the complexity of these models and the

Table 6. Optimized P_t values, number of fish recaptured at least once, standard deviation (SD) of r_j multipliers, and Kolmogorov–Smirnov (KS) test of normality results for each of 13 growth experiments conducted in the Pine River watershed, 2001 and 2002.

Branch	Site	No. of fish recaptured	r_j multiplier SD	P_1	P_2	KS *p*
2001						
East	A	36	0.253	0.166		<0.01
South	F	33	0.187	0.168	0.083	>0.15
South	L	18	0.235	0.113	0.079	>0.15
South	G	41	0.200	0.119	0.055	>0.15
West	M	31	0.207	0.155	0.082	>0.15
West	I	22	0.202	0.152	0.086	>0.15
Average			0.214	0.146	0.092	
2002						
Backus	K	19	0.187	0.271	0.115	0.10
East	A	61	0.176	0.275	0.145	>0.15
East	B	23	0.122	0.286	0.135	>0.15
Main	D	29	0.243	0.318	0.097	>0.15
South	E	11	0.152	0.237	0.126	>0.15
South	F	36	0.219	0.230	0.119	>0.15
West	M	45	0.226	0.295	0.101	<0.01
Average			0.189	0.273	0.120	

large number of parameters that are often fixed from laboratory studies or assumptions based on other species or life stages, there is considerable confounding between estimates of consumption and these other parameters. As such, we concur with the recommendation of Chipps and Wahls (2008) that estimates of the proportion of maximum consumption (*P*), which is related to consumption, be treated with caution, particularly when used as absolute measures rather than relative measures. Deviations in parameters such as energy content of predators and prey and the proportion of prey that is indigestible from actual values can result in substantial bias in estimates of total consumption or *P*, for example. Our use of a standard weight equation may likewise result in some inaccuracy in estimates of *P*.

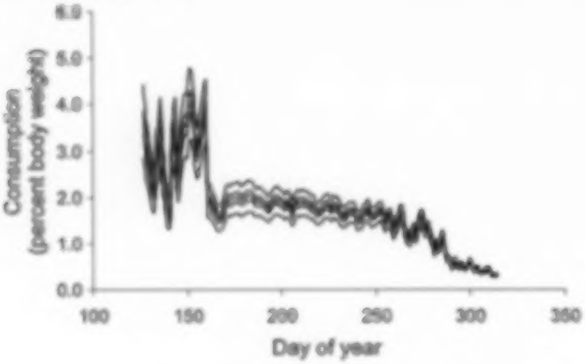
In principle, parameters beyond consumption could be estimated from field data on free-ranging fishes. In practice, however, the high degree of confounding would not allow for unique estimates of multiple parameters simultaneously. As shown by Rennie et al. (2005), additional data such as contaminant accumulation rates are typically needed to estimate parameters beyond consumption. With the approach that we present in this paper, it would be possible and practical to statistically estimate parameters in addition to *P* when such auxiliary data are available.

One of the unique aspects of this study is the inclusion of a set of random-effect multiplicative factors (i.e., r_j) that allow for the estimation of variability among free-ranging individuals. Laboratory studies have previously shown that bioenergetic processes vary among individuals (e.g., Tyler and Bolduc 2008) where experimental conditions can be maintained and replicate measurements can be taken on individual fish. To our knowledge, ours is the first application of a statistical estimator of individual variability in a field-based bioenergetics model of fish. There are limitations to this method, however. First, the data structure itself may limit the application of our multiplier model. We note that models structured with a fish-specific r_j exactly fit the growth for fish with two size observations and one P_t and for fish with three observations and two P_t. This can lead to situations in which the model is overparameterized, leading to difficulties in interpretation.

Another important consideration is the interpretation of the r_j for individual fish. Within our modeling structure, r_j is implemented as a multiplier to P_t, implying that r_j represents variability in proportion of maximum consumption rates. In actuality, variation in r_j represents the net balance among multiple factors that lead to differences in growth rate among fishes at the same site. For example, genetic variation among individuals may lead to variation in an individual's basal respiration rate or maximum consumption rate (Tyler and Bolduc 2008). Another source of variation in growth among individuals is that food resources and activity costs are heterogeneous within stream reaches and individual fish occupy habitats of varying quality (e.g., Fausch 1984). Likewise, variation in prey energy density or the energy density of individual steelhead is potentially reflected in variability in r_j, as well as other aspects of fish biology such as individual variation in behavior. Thus, variation in r_j incorporates all of these influences into a single aggregated parameter.

Because we parameterized the bioenergetics model using *P* as the primary free parameter, as has been commonly done in prior studies (e.g., Railsback and Rose 1999), prey consumption rates are confounded with several factors, including temporal variation in temperature and fish size. As such, it is difficult to directly determine how consumption varies over time or among fish. The bioenergetics modeling framework readily allows predictions of individual consumption rates (as illustrated in Fig. 8) based on the structure imposed by using *P* as the free parameter. Nonlinearities in the bioenergetic relationships can lead to biologically important differences in estimated total con-

Fig. 8. Trends in predicted consumption rate, expressed as percent body weight per day, for five simulated fish from the South Branch of Pine River in 2001.



sumption if individual variation is ignored. For example, total consumption over the course of the season by a "typical" fish at the campground site in 2001 with a r_j multiplier of 1.0 was 5805 g. Estimates of consumption by fish with r_j multipliers of 0.7 and 1.3 (i.e., equidistant from 1.0 and averaging 1.0) were 3268 g and 9251 g, respectively, and averaged 6269 g, which was 7.8% greater than for the average fish.

We illustrate how the results of the multiplier model can be incorporated into an individual-based model framework (Fig. 8). Essentially, an individual-based model can be run whereby each fish is given a random multiplier drawn from a normal distribution with a mean of 1.0 and the standard deviation estimated from the experiment. For these simulations, we emphasize that it is important to maintain an appropriate time scale (here, the entire growth season) when incorporating estimates of r_j in individual-based models. Thus, when using these random effects in a individual-based model, the random r_j chosen must be held constant across the entire growth season and not allowed to vary randomly on shorter time steps (e.g., daily). The consequence of randomly selecting r_j daily is to greatly reduce the amount of growth variation among individuals (D.B. Hayes, unpublished data), thus misrepresenting the true situation in the field.

In summary, bioenergetics models have seen wide application across a variety of species to better understand the influence of environmental conditions on the consumption and growth of fish populations. The approach that we present in this paper now allows the application of these models to situations in which the focus is on individual fish growth. Such insights are increasingly important given the recognition of the impact of individual phenotypic variation in the dynamics of ecological systems. Moreover, the model that we apply uses statistical methods both to fit model parameters to the data and to formally test models of varying complexity. Our hope is that this extension to bioenergetics modeling opens up new insights and opportunities into the investigation of fish ecology.

Acknowledgements

We thank T. Coon, M. Jones, and G. Mittelbach for guidance on model development and reviews of earlier drafts of this manuscript. We also are grateful to T. Riley, B. Bellgraph, E. McCoy, B. Elkington, M. Klungle, K. Mueller, M. Faulk, K. Mann, A. Thomson, K. DeGrandchamp, and A Shultz for invaluable assistance with fish tagging and sampling. This project was supported in part by funds from the Fisheries Division of the Michigan Department of Natural Resources, US Department of Agriculture McIntyre–Stennis Program, Great Lakes Fishery Commission, and the Schrems West Michigan Chapter of Trout Unlimited.

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Modelling fishing-induced adaptations and consequences for natural mortality

Christian Jørgensen and Øyvind Fiksen

Abstract: When trade-offs involving predation and mortality are perturbed by human activities, behaviour and life histories are expected to change, with consequences for natural mortality rates. We present a general life history model for fish in which three common relationships link natural mortality to life history traits and behaviour. First, survival increases with body size. Second, survival declines with growth rate due to risks involved with resource acquisition and allocation. Third, fish that invest heavily in reproduction suffer from decreased survival due to costly reproductive behaviour or morphology that makes escapes from predators less successful. The model predicts increased natural mortality rate as an adaptive response to harvesting. This extends previous models that have shown that harvesting may cause smaller body size, higher growth rates, and higher investment in reproduction. The predicted increase in natural mortality is roughly half the fishing mortality over a wide range of harvest levels and parameter combinations such that fishing two fish kills three after evolutionary adaptations have taken place.

Résumé : Lorsque les compromis entre la prédation et la mortalité sont perturbés par les activités humaines, on s'attend à ce que les comportements et les cycles biologiques changent, ce qui affecte les taux de mortalité naturelle. Nous présentons un modèle général de cycle biologique dans lequel trois relations courantes relient la mortalité naturelle aux traits du cycle biologique et au comportement. D'abord, la survie augmente en fonction de la taille corporelle. Ensuite, la survie diminue en fonction du taux de croissance à cause des risques reliés à l'acquisition et l'allocation des ressources. Enfin, les poissons qui investissent beaucoup dans la reproduction ont une survie réduite à cause des coûts associés aux comportements reproducteurs ou à la morphologie qui réduisent le succès de l'évitement des prédateurs. Le modèle prédit un taux de mortalité naturelle accru comme réaction adaptative à la récolte. Cela élargit les modèles antérieurs qui ont montré que la récolte peut produire des tailles corporelles réduites, des taux de croissance plus élevés et un investissement accru dans la reproduction. L'accroissement prédit de la mortalité naturelle correspond en gros à la moitié de la mortalité due à la pêche sur un large éventail d'intensités de récoltes et de combinaisons de paramètres, de telle sorte que la pêche de deux poissons en élimine trois une fois que les adaptations évolutives ont eu lieu.

[Traduit par la Rédaction]

Introduction

Current fishing levels have been suggested to induce evolutionary changes in fish stocks on decadal time scales (Law and Grey 1989; Rijnsdorp 1993; Jørgensen et al. 2007). A reduction in age and size at maturation has been the most widely studied response to increased fishing mortality, partly because suitable data exist (Dieckmann and Heino 2007), but changes in growth rate (Ricker 1981) and reproductive investment (Yoneda and Wright 2004) have also been documented. In this paper, we turn the focus to natural mortality rates. Many of the trade-offs that shape behaviour and life histories involve predation risk. When fish behaviour and life histories change, it is likely that natural mortality rates might change too. Here we investigate the potential consequences of fishing-induced adaptations for natural mortality rates in a model with three general relationships that link survival with life history traits and behaviour.

The first relationship describes how predation typically declines with size in marine systems (Peterson and Wroblewski 1984), scaling with weight as $W^{-0.25}$ or length as $L^{-0.75}$. The probability of encountering a predator large enough to engulf a prey decreases with size. Theoretical approaches that combine principles of mass balance, the allometric scaling of metabolic rate, and mechanics of predation lead to emergent size spectra where predation scales as $W^{-0.25}$ (Sheldon et al. 1972; McGurk 1986; Andersen and Beyer 2006). When maturing at a smaller size, fish spend more of their life under higher levels of size-dependent predation.

Second, foraging often involves exposure to risk when searching for food (Lima and Dill 1990). Schooling species trade between foraging and safety through their position within or distance from the group (Pitcher and Parrish 1993). Fish are mainly visual feeders, and seeing well normally implies increased risk of being seen. Pelagic organ-

Received 9 September 2009. Accepted 23 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 11 June 2010.
J21401

Paper handled by Associate Editor Eric Taylor.

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Can. J. Fish. Aquat. Sci. 67: 1086–1097 (2010) doi:10.1139/F10-049 Published by NRC Research Press

isms can easily modulate risk exposure by vertical migration from well-lit surface layers to dark, deep waters, thus trading foraging success against survival (Clark and Levy 1988). Similar trade-offs between growth and survival can also arise through physiological mechanisms. For example, Atlantic silversides (*Menidia menidia*) that ingest larger meals are poorer swimmers and experience higher rates of predation (Billerbeck et al. 2001; Lankford et al. 2001). The reason is that they use more of their oxygen budget for digestion and therefore have less aerobic scope available for escaping predators (Arnott et al. 2006). This cost accelerates with growth rate (Munch and Conover 2004). Growth can also be enhanced by allocating less resources to processes such as immune defence (Lochmiller and Deerenberg 2000), with negative implications for survival. These diverse growth-related costs all ultimately compromise survivorship.

The third relationship is a trade-off between reproductive investment and survival. Reproduction often involves display behaviour that may attract predator attention (Endler 1987), mate search that may lead to more frequent predator encounters (Kiørboe 2008), and development of large gonads that may reduce swimming performance (Ghalambor et al. 2004).

Natural selection is acting on these relationships simultaneously so that foraging behaviour, growth strategies, sexual maturation, and reproductive investment evolve in concert. Because harvesting essentially is just another form of predation, fishing is expected to cause evolutionary changes in the optimal life histories. Because all the trade-offs described above involve mortality and predation, we expect there to be consequences also for natural mortality. In other words, should fish take higher risks in their foraging behaviour, growth strategy, or reproductive effort now that their longevity is greatly reduced by fishing? Our aim is to predict the expected change in natural mortality rates using life history theory to model fishing-induced evolution.

Materials and methods

We use a relatively simple state-dependent life history model in which optimal life histories are found by optimization. The central mechanisms are energy acquisition and energy allocation, with corresponding decision variables being the risk-taking of the growth strategy (trading off growth versus survival) and energy allocation between somatic growth and reproduction (trading off growth versus reproduction and reproduction versus survival). In the following description, capital letters such as W (weight) and L (length) denote functions, whereas lowercase letters such as $w(a)$ and $l(a)$ denote specific values that these functions take, here at the particular age a.

Life history assumptions

We assume that available resources (R, in equivalents of body mass (grams·year^{-1})) increase with somatic body mass (W, grams) as

$$(1) \qquad R = h \cdot W^b$$

where b (dimensionless) is an allometric scaling exponent. The parameter h adjusts net available resources up and down depending on the individual's growth strategy (φ, dimensionless). High φ may represent more risky foraging behaviour (Lima and Dill 1990), elevated digestion at the cost of the ability to escape predators (Billerbeck et al. 2001; Lankford et al. 2001), or reduced allocation to, e.g., immune defence (Lochmiller and Deerenberg 2000). In particular, we assume that h increases asymptotically up to h_{max}:

$$(2) \qquad h = \frac{h_{max} \cdot \varphi}{h_{1/2} + \varphi}$$

Here $h_{1/2}$ (dimensionless) is the half-saturation constant. This is analogous to a Holling type II functional response, but where higher risk acceptance of φ leads to higher growth rates through the growth coefficient h in eq. 1. It is reasonable to parameterise increased resource availability in the environment as a lower value for the half-saturation constant $h_{1/2}$ so that a higher growth could be achieved at lower levels of risk acceptance. Following that interpretation, the asymptote h_{max} would be the physiological maximum growth rate with ad libitum food and when no trade-offs that involve predation or mortality would constrain growth rates. The φ is modelled as state-dependent, so that the optimal φ is found independently for each age a (years) and body length l (cm), i.e., $\varphi(a, l)$.

A second state-dependent strategy, also depending on age a and length l, describes resource allocation $\alpha(a, l)$, which divides R between somatic growth and gonads (G, grams):

$$(3) \qquad dW/dt = (1 - \alpha) \cdot R$$

$$(4) \qquad dG/dt = \alpha \cdot R$$

Length (L, cm) is isometrically related to weight, $W = kL^3$. Gonads are interpreted in a broad sense to include, e.g., mating behaviour and spawning migrations. Growth, mortality, and the accumulation of gonads were modelled as continuous processes, whereas spawning takes place once per year (at the year's end). Conceptually, our approach falls within the class of energy allocation models (e.g., Roff 1983; Kozlowski 1991), and we separate between irreversible structural mass and reversible reserves and gonads as done also by, e.g., de Roos and Persson (2001) and Jones et al. (2002). The growth model is similar to that developed by Lester et al. (2004) and Quince et al. (2008), except that we use an exponent b that deviates from 2/3.

Mortality is split into five categories (all with unit year^{-1}). (*i*) There is a fixed base level of size-independent mortality, M_{fixed}. (*ii*) Size-dependent predation (Peterson and Wroblewski 1984; Andersen and Beyer 2006) declines with body size as $M_{predation}(L) = cL^{-d}$. (*iii*) The φ leads to increased growth-related mortality M_{growth}. Having high φ leads to increased predation mortality: there is thus a trade-off between net ingested resources and survival. We assume that this mortality follows the same size dependence as predation, $M_{growth}(\varphi, L) = \varphi M_{predation}(L)$. (*iv*) Reproduction-related mortality $M_{reproduction}$ increases with reproductive investment and follows the same size dependence as predation. We model this as $M_{reproduction}(Q, L) = M_{predation}(L) \cdot (Q/q_{ref})^p$, where q_{ref} is a reference value at which the mortality from reproduction equals the size-dependent predation component, i.e., at which $M_{reproduction} = M_{predation}$. For $p > 1$, mortality rate accelerates with increasing reproduction as, e.g., observed in guppies (Ghalambor et al. 2004). (*v*) Finally, we added fishing mortality (F), which may depend on body length. Total mortality rate (Z, year^{-1}) is then $Z(\varphi, Q, L) = M_{\mathrm{fixed}} + M_{\mathrm{predation}}(L) + M_{\mathrm{growth}}(\varphi, L) + M_{\mathrm{reproduction}}(Q, L) + F$. Annual survival probability (S) is thus found as

$$(5) \qquad S(A) = \exp\left(-\int_{t=A}^{A+1} Z\,\mathrm{d}t\right)$$

Optimization and implementation

As fitness measure, we used R_0, defined as the expected lifetime production of gonads, i.e., the sum of G over all ages discounted by survival probability until that age. We used the optimization technique of dynamic programming (Houston and McNamara 1999; Clark and Mangel 2000) to find optimal trajectories of growth, survival, and reproduction. This is a process-based approach working from mechanisms to population-level patterns, in contrast to Bayesian and other statistical approaches that identify patterns suggestive of mechanisms at work beginning with data. In our case, these trajectories are specified by the state-dependent strategies $\varphi(a, l)$ and $\alpha(a, l)$ for all combinations of ages a (in steps of 1 year) and lengths l (in steps of 1 cm) that maximize R_0. Maximum age ($a_{\max}$) was set to 30 years after which all individuals die, and maximum length ($l_{\max}$) was set to 200 cm ($a_{\max}$ and $l_{\max}$ are technical limits in the algorithm and should be well beyond the ages and sizes regularly observed to avoid boundary effects).

Equations 3, 4, and 5 allow us to relate body mass at the start of the year to body mass, gonad mass, and survival at the end of the year. The continuous time integrals were solved numerically by dividing each year into J discrete intervals for which size, gonads, and mortality were updated, while the strategies $\alpha(a, l)$ and $\varphi(a, l)$ remained fixed within each year. In the results shown here, we used $J = 24$ (two time steps per month), at which the strategies and individual trajectories were indistinguishable from a (computationally slow) test simulation of $J = 1000$ (full discrete time equations are given in Appendix A). In the current formulation of the model, we did not include seasonal cycles.

Given $w(a)$ and the strategies $\alpha(a, l)$ and $\varphi(a, l)$, we find $w(a + 1)$ from which length at the next age is found as

$$(6) \qquad l' = \left(\frac{w(a+1)}{k}\right)^{1/3}$$

As optimization criterion for fitness, we define $v(a, l)$ as the expected future production of gonads until $a_{\max}$ of an individual of length l at age a. By assuming that individuals have no future fitness at $a_{\max}$, the dynamic programming equation shows how reproductive value ($v(a, l)$) at age a and length l can be found through recursive iterations:

$$(7) \qquad v(a, l) = \max_{\alpha, \varphi}\{s(a)[g(a+1) + v(a+1, l')]\}$$

Because we use optimization, the predicted life histories are evolutionary end points (examples of optimal strategies are shown in Supplemental Fig. S1[3]). The rest of the results shown are from simulations of an individual following the optimal strategies over its lifetime (initialised at 16 cm and 1 year). In the results, we focus on traits that could be quantified in empirical studies; rather than the hidden process variable α, we therefore present reproductive investment as the gonado-somatic index $Q = G/(W + G)$ (dimensionless).

Parameterisation

We manually tuned the parameters for mortality so that total natural mortality without fishing was 0.2 at $l = 100$ cm for an immature fish and with $M_{\mathrm{predation}}$, M_{growth}, and $M_{\mathrm{reproduction}}$ being of equal magnitude. Resulting parameter values were $b = 0.7$, $d = 0.75$, $k = 0.01$, $c = 2$, $h_{\max} = 5.5$, $h_{1/2} = 0.10 \cdot h_{\max}$, $q_{\mathrm{ref}} = 0.25$, $p = 2$, and $M_{\mathrm{fixed}} = 0.067$. The model's sensitivity to these values was tested varying each parameter value and reporting the effect on the main prediction: the increase in natural mortality as a function of the imposed harvesting mortality. We also tested how a minimum landing size in the fishery or constraints on maximum reproductive investment affected the model's predictions. The sensitivity analysis is shown briefly herein (further details can be found in Supplemental Fig. S2[3]).

Results

The model predicts that natural mortality rates increase with increased harvesting. This result extends the standard expectation for life history responses to fishing, namely, that fish will mature earlier and at smaller sizes (e.g., Law and Grey 1989). Natural mortality increases because life histories evolve towards smaller size, more risky foraging, and elevated reproduction (Figs. 1, 2).

Mortality increases most after maturation

The age-specific response suggests that the effect of these adaptations is strongest from maturation onwards (Fig. 3) for two reasons. First, higher reproductive investment slows growth and fish therefore spend the remainder of their life at a smaller size with higher size-dependent predation rates. Second, intensified reproduction mean larger gonads, which incur reduced survival. As maturation age declines, these effects begin earlier in life.

Fishing two fish kills three

Natural mortality rates increase almost proportionally with harvest rates up to 0.5 year^{-1} and from then on somewhat slower than proportionally (Fig. 4*a*). The extra mortality rate is approximately half the harvest rate. Fishing two fish thus kills three, once life histories and behaviour have adapted to the new harvest regime.

Trends in productivity may mask adaptation

Fishing reduces population size and may weaken density-dependent competition for food. If food abundance increases (in the model by lowering the value of $h_{1/2}$), fish may grow faster and accept less risk through the mechanisms described above, with the overall effect of reducing natural mortality (Fig. 4*b*). However, optimal strategies still accept more natural mortality as harvest rates go up (moving along each line

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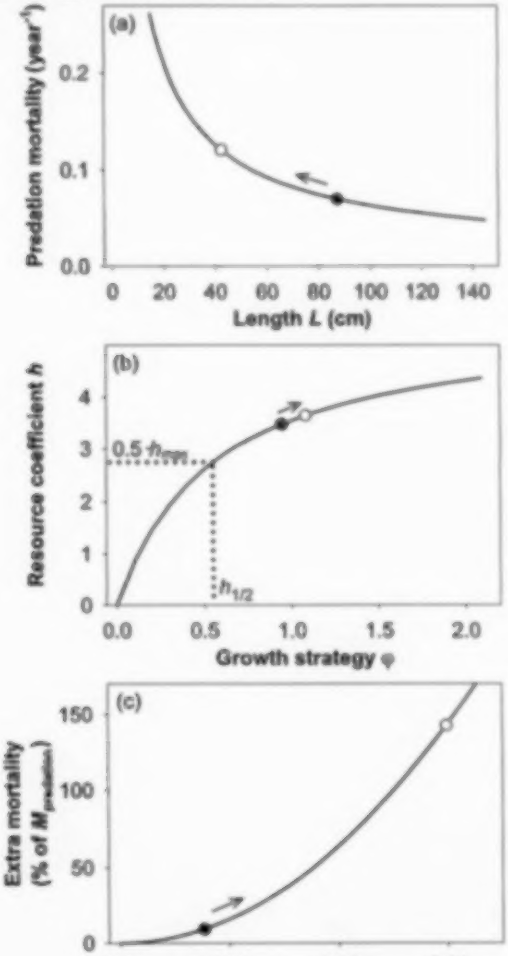
[3] Supplementary data for this article are available on the Journal web site (http://cjfas.nrc.ca).

Fig. 1. Central assumptions of a life history model that quantifies fisheries-induced effects on natural mortality rates. (*a*) Size-dependent predation mortality declines with size, $M_{\text{predation}} \propto W^{-0.25} \propto L^{-0.75}$. Symbols denote size at 12 years for the optimal strategy under no fishing (solid circle) and when harvest rate is 0.5 year^{-1} (open circle). (*b*) The assumed trade-off between growth strategy (φ) and the coefficient h of the net resource availability function. The values for the half-saturation constant ($h_{1/2}$) and half the asymptotic level ($0.5 \cdot h_{\max}$) are indicated with dotted lines. Symbols (as in *a*) denote the optimal growth strategy of a 40 cm fish. (*c*) The relationship between extra predation mortality and reproductive investment, quantified as the gonado-somatic index (Q, gonad mass divided by total mass). Symbols (as in *a*) illustrate optimal strategy for a 12-year-old fish.



towards the right). Thus, although natural mortality may fluctuate depending on density dependence and environmental conditions, life history adaptations to fishing will make it fluctuate at a higher level.

A gradual increase in food abundance may reduce natural mortality rate as there is less need for risky foraging and the abundant food may allow larger body sizes. Thus, higher food levels (a move from the broken to the continuous to the dotted lines in Fig. 4*b*) could mask concurrent evolution towards higher natural mortality rates (moving from left to right on each line in Fig. 4*b*). However, if food level fell to original levels, the new adaptations towards riskier behaviour and smaller size would imply rising natural mortality rates. Furthermore, evolutionary changes in behaviour and life histories are likely to have slower dynamics than changes in food abundance. The evolved higher natural mortality rates may therefore prevail for some time even if fishing pressure were reduced. This effect is consistent with the surprisingly slow recovery of many fish stocks that have collapsed from overfishing (Hutchings 2000).

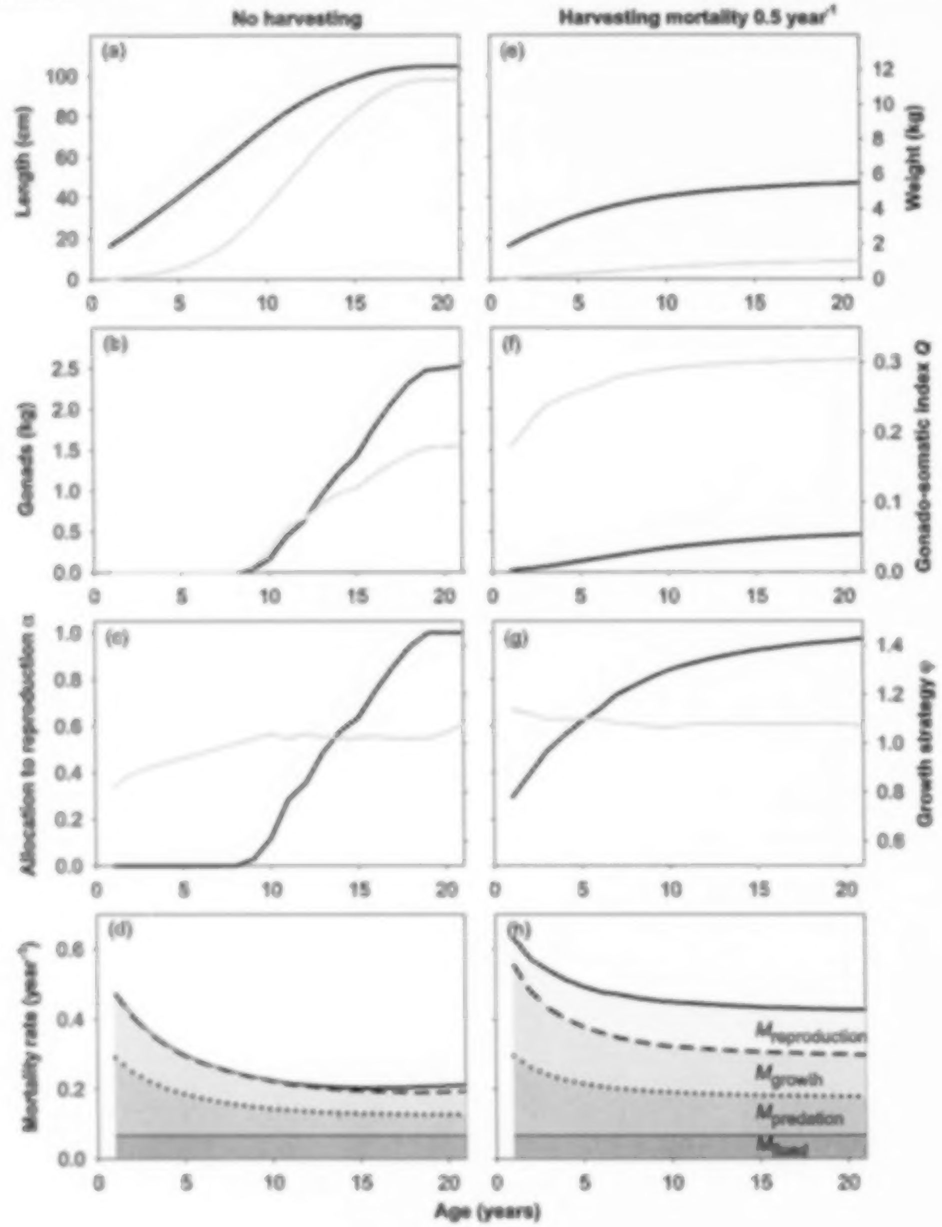
Sensitivity analysis

The prediction of increased natural mortality is robust to variation in parameter values (Fig. 4*c* and Supplementary data[3]). We checked sensitivity by letting central parameters vary from the standard configuration. Although this may alter life history traits such as age, maturation, and growth rates considerably, the response in terms of increased natural mortality rates was much less variable. Almost all trade-offs in the model, and many in nature, involve mortality risk either directly or indirectly. Under fishing, the key life history response is to increase current reproduction (while still alive) at the expense of future reproduction (which may never be realised). Although this can happen through many routes, e.g., increased acquisition, reduced size at maturation, or increased reproductive investment, the common consequence is that survival is sacrificed.

The model is most sensitive to variation in the parameter p, which describes how quickly mortality increases as reproductive investment increases. Especially, the model predicts dramatic consequences for natural mortality if even a small reproductive investment would inflict mortality costs ($p = 1$; Fig. 4*c*). Mechanisms that scale reproduction mortality approximately linearly with reproductive investment likely include mate search, if more intense reproduction requires more matings, and prolonged presence at the spawning grounds, for example, in batch spawners that need to mature batches of eggs successively (Kjesbu et al. 1996). The parameter value of $p = 2$ in the standard scenario leads to less dramatic predictions and takes into account that several survival costs are initially low but accelerate with the intensity of reproduction. Examples of such costs include hydrodynamic drag of bulky body shapes and reduced swimming performance due to competing metabolic needs (Ghalambor et al. 2004; Munch and Conover 2004).

Other parameters to which the model is sensitive are as follows. (*i*) If survival costs only kick in when gonads are large, the model predicts that natural mortality will increase less ($p = 3$). (*ii*) If there is a minimum size limit in the fishery, life histories may evolve to spend more time in size windows of lower mortality. We modelled fisheries selectivity as $Y(L) = [1 + \exp(-u(L - L_{50}))]^{-1}$, with $u = 0.1$ (steepness) and $L_{50} = 40$ (cm) being the length at which there is 50% probability of being harvested; total fishing mortality for a given length is then $F(L) = F_{\max} \cdot Y(L)$, where $F_{\max}$ is the fishing mortality at sizes where the selectivity curve $Y(L)$ approaches 1 (further minimum size limits are reported

Fig. 2. Individual trajectories of (*a* and *e*) growth in terms of length (bold line, left axis) and weight (shaded line, right axis), (*b* and *f*) reproduction quantified as gonad weight (bold line, left axis) and gonad-somatic index (shaded line, right axis), (*c* and *g*) optimized strategy for allocation (bold line, left axis) and growth strategy (shaded line, right axis), and (*d* and *h*) acquired mortality found through forward simulation of state-dependent life history strategies. The left column (*a*–*d*) is optimized for no harvest and the right column (*e*–*h*) represents a harvest rate of 0.5 year^{-1}.

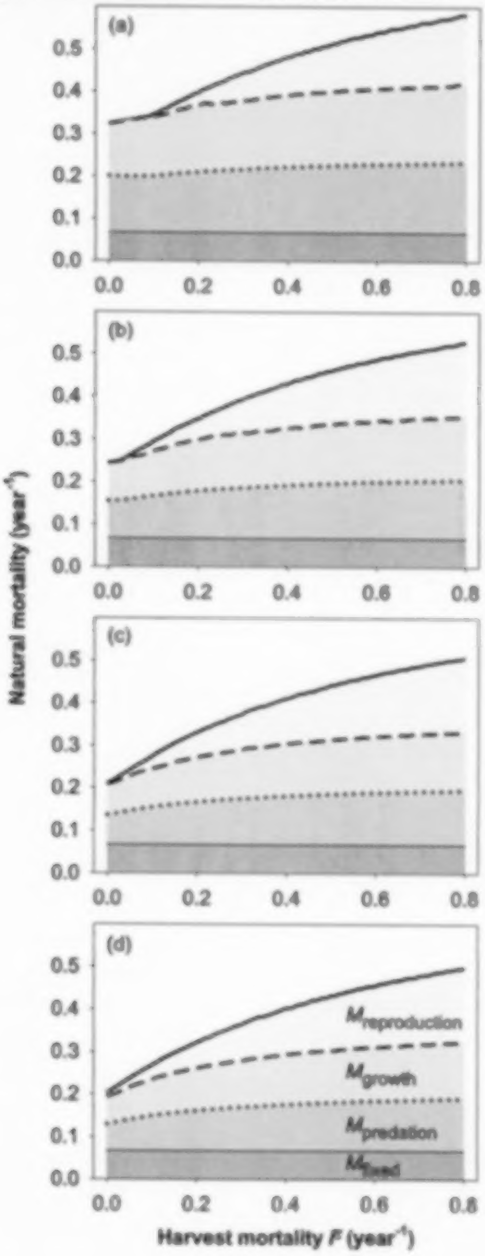


in Supplemental Fig. S2 (*h*), available online[3]). (*iii*) If the magnitude of adaptations are constrained by imposing limits in the model, then the extra natural mortality may reach a ceiling. We imposed a constraint so that the gonado-somatic index (Q) cannot exceed a value of 0.25, which led the extra natural mortality to level out at around 0.1 from $F = 0.2$ onwards.

Discussion

Over the last century, industrial fishing has (*i*) changed the marine community composition towards smaller-sized species at lower trophic levels (Pauly et al. 1998), (*ii*) shifted population distributions towards younger and smaller fish, and (*iii*) caused evolutionary change in life his-

Fig. 3. Age-dependent life history responses to harvesting mortality. The four components of natural mortality rate are shown for ages (*a*) 4, (*b*) 8, (*c*) 12, and (*d*) 16 years for optimal life history strategies under increasing harvesting pressure *F* (*x* axis).



tory traits towards earlier maturation at smaller sizes (reviewed in, e.g., Dieckmann and Heino 2007; Jørgensen et al. 2007; Allendorf et al. 2008). In this paper, we focus on the evolutionary implications and used a model to predict that natural mortality will increase as a consequence of earlier maturation at smaller size, higher risk acceptance in foraging behaviour and growth strategies, and intensified reproductive effort.

The changes predicted by our model are rather dramatic. With no harvesting, it predicts a fish life history that matures at age 9 with a gonado-somatic index of 10%–20% and a length at age 12 of 90 cm. In contrast, the predicted life history adapted to a harvesting rate of 0.5 year^{-1} matures at age 1, the gonado-somatic index exceeds 25% already at age 5, and the fish is only 40 cm when it is 12 years old. This difference is comparable with that between a cod and a herring, and it might be unlikely that an evolutionary change of this magnitude will take place in the foreseeable future. To interpret the model, it may therefore help to think of the trade-offs that we assumed as representative of classes of mechanisms. A general problem with models is that they allow for only a few evolving traits, whereas in real species, all traits can evolve simultaneously. In a model, the total selection pressure thus needs to be absorbed by these few traits, whereas there are more degrees of freedom for evolution in the wild. For example, we assumed that increased investment in reproduction would lead to a higher gonado-somatic index, with increased mortality through predation as a consequence. Translated to the wild, this does not necessarily imply that fish will have larger gonads — there are several ways in which fish can achieve higher reproductive investment. For example, the fish may invest more in spawning by prolonging the time used for spawning activity, spend more time and energy migrating between feeding and spawning areas, or engage more in display and mating behaviour. These kinds of changes can all have similar implications for predation rates as our assumed trade-off. An alternative mechanism that links reproduction and survival could be starvation after severe depletion of energy stores due to spawning. In general, starvation tolerance increases with size, which would require a different formulation for the trade-off between reproduction and survival than what we have implemented.

Survival depends on the mortality level (the rate) and the duration of exposure to that mortality. When external mortality (fishing) goes up, adaptations that shorten exposure to that mortality will increase survival more than before, and that extra benefit can be achieved through several trade-offs through which fish accept higher mortality rates to reproduce more earlier in life.

Assumption: predation declines with size

One of our key assumptions is that predation mortality declines with body length according to an allometric scaling relationship of the type $M \propto L^{-d}$. Based on comparisons across species covering a wide size range (Brown et al. 2004), we used a value of $d = 0.75$. However, these types of scaling relationships between species are not necessarily good descriptors of the scaling relationship within a species, which is what we model (Tilman et al. 2004). When we therefore tested for the sensitivity to this central parameter, the model's predicted increase in natural mortality rate was more severe for higher values of d. Even with $d = 0$, however, the model predicted that natural mortality would increase by roughly 25% of the fishing mortality. The value $d = 0$ means that predation does not depend on size, which describes the unlikely situation that a 2 m tuna has the same

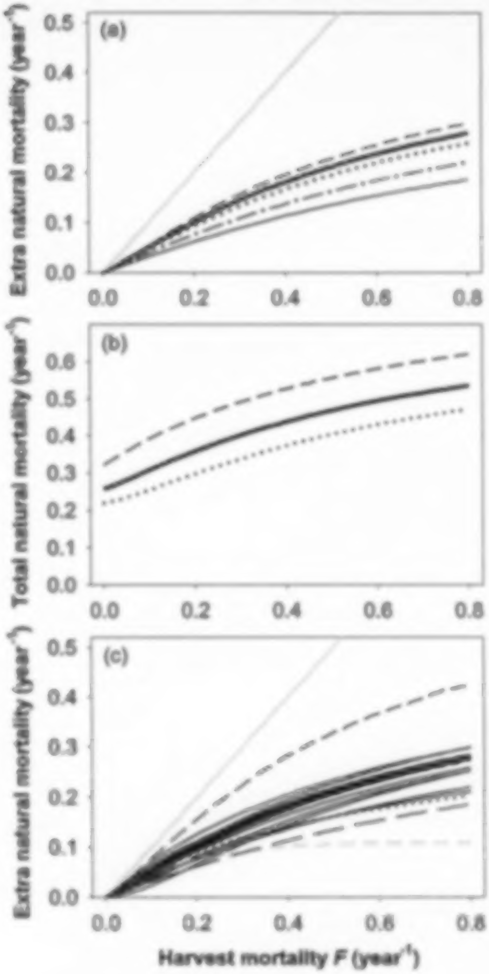


Fig. 4. Total effect on natural mortality in relation to harvest rate, averaged over the first 20 years of life. (*a*) The thick continuous line uses the standard assumption that $d = 0.75$ in the allometric scaling of predation mortality with size, $M \propto L^{-d}$. Sensitivities to this central parameter are shown with the broken ($d = 0.9$), dotted ($d = 0.6$), dash–dotted ($d = 0.3$), and thin lines ($d = 0$); natural mortality was kept constant for a 60 cm fish. The shaded line denotes 1:1. (*b*) Total natural mortality (fishing mortality is excluded) when varying the half-saturation constant $h_{1/2}$ of the growth strategy – resource intake relationship from Fig. 1*b*. The continuous line is for standard parameters. The dotted line has an initial slope that is twice as steep ($h_{1/2} = 0.05 \cdot h_{max}$), representing a situation with abundant and accessible food. Optimal strategies still have higher natural mortality when there is harvest but less than in the standard situation. For the broken line, the initial slope is shallower, representing food shortage ($h_{1/2} = 0.20 \cdot h_{max}$). Increasing food will reduce mortality from broken to continuous to dotted lines. (*c*) Sensitivity analysis (more detailed figures are shown in the online supporting information[3]). The extra natural mortality that results from standard parameters is shown in thick continuous line. In many cases, predictions do not change notably when parameters are varied; this can be seen as the cluster of thin continuous lines around the main prediction (from top to bottom as their effect is at $F = 0.80$: $h_{1/2} = 1.1$; $d = 0.90$, $h_{max} = 4.5$, $q_{rel} = 0.35$, [thick line with standard parameters], $q_{rel} = 0.15$, $b = 0.65$, $h_{max} = 6.5$, $d = 0.60$, $h_{1/2} = 0.28$, $b = 0.75$, $d = 0.3$, $p = 3$). Notable exceptions, discussed in the text, are as follows: $p = 1$ (short-dashed line); a minimum size limit in the fishery of 40 cm (dotted line); $d = 0$ (long-dashed line); and a constraint so that gonads the gonado-somatic index Q cannot exceed a value of 0.25 (shaded broken line).

probability of being eaten per unit time as a 10 mm tuna larvae. However, fish larvae generally have predation rates in the range of 0.05 to 0.5 day^{-1}, or 18 to 180 year^{-1} (McGurk 1986), compared with the standard assumption of adult mortality rate of around 0.2 year^{-1} generally used in fisheries science (Jennings et al. 2001, p. 203). Thus, some sort of decline in predation mortality seems to be supported by data. The exact value of d changes our model's quantitative predictions, but the qualitative pattern is persistent, namely increased natural mortality rate as life histories adapt to fishing.

Example: Canadian cod stocks

The potential implications of evolutionary changes in natural mortality rate can be illustrated with an example from the literature. Many Canadian stocks of Atlantic cod (*Gadus morhua*) were heavily harvested prior to their collapse throughout the 1980s. A fishing moratorium was implemented in the early 1990s, although some fishing continued (Shelton et al. 2006). Despite these severe management restrictions, the stocks have failed to recover. Using survey data from the southern Gulf of St Lawrence stock, Swain and Chouinard (2008) found a temporal trend towards higher natural mortality, with the estimate for recent years being 0.6 year^{-1}, three times higher than the presumed value prior to 1980. Swain and Chouinard (2008) write that "causes of the apparent increase in *M* are unknown." Applying the insights from our model, however, it seems that several of the mechanisms that lead to higher natural mortality in our model have been observed in the Canadian cod stocks. First, the driver of change in our model is fishing pressure, and fishing mortality rate on these stocks have typically been in the range of 0.3 to 1.0 year^{-1} for decades and, furthermore, increasing in the years leading up to the moratorium (Myers et al. 1997). Second, size and age at maturation have declined in most of the stocks, presumably driven by fishing-induced evolution (Olsen et al. 2005). In our model, high fishing mortality made it optimal to mature early while still alive. Third, size at age has declined for postmature fish, presumably as a response to fishing, at least in the southern Gulf of St. Lawrence stock (Sinclair et al. 2002; Swain and Chouinard 2008). This is again consistent with the model's predictions: early maturation redirects resources towards reproduction and will therefore decelerate growth so that, for all ages after maturation, the fish are of smaller body size. Increasing reproductive investment would have similar effects in reducing size at age of postmature fish (Heino et al. 2008). Fourth, recruitment per unit biomass is higher in recent years than prior to the 1970s (Swain and Chouinard 2008). This is also in accordance with our model, which predicted that reproductive investment would go up.

Thus, all these patterns are consistent with the fishing-induced effects modelled in our analysis, where riskier energy acquisition, earlier maturation, and elevated reproductive investment all bring about higher natural mortality as an adaptive response to cope with increased fishing mortality. It is important to emphasise that there are other nonexclusive explanations for most of these patterns. Many elements of these Canadian ecosystems have changed, and both size-selective fishing and density dependence have been variable in the Gulf of St. Lawrence stock in particular (Sinclair et al. 2002; Swain et al. 2007, 2008). The general lack of recovery of cod in the area has been attributed to, e.g., discarding of young fish (Myers et al. 1997) and regime shifts (Petrie et al. 2009). The increased recruitment in recent years may be due to released density dependence when the spawning population is small, as is commonly observed in many fish stocks, but there has also been a general increase of small fish species in this area that might reflect reduced abundance of large predators (Benoît and Swain 2008). The timing of events may also suggest a role for other factors: for southern Gulf of St. Lawrence cod, maturation age decreased in the 1960s and 1970s, before the decline in size at age, which took place in the 1970s and 1980s. It could still be that maturation age changed first but that an effect of decelerated individual growth only occurred later, in response to an increase in reproductive investment. However, evidence suggests that fisheries size selectivity and density-dependent growth processes have also contributed (Sinclair et al. 2002; Swain et al. 2007).

Our model provides new hypotheses consistent with observed changes in these cod stocks, but the change may also be explained by several alternative ecological mechanisms. It will be interesting to see from future analyses the role that the various hypotheses have played relative to each other. Often these explanations do not exclude each other, and probably ecological and evolutionary processes have worked in concert to produce the final outcome.

Also, we are not aware of any studies that document increased risk-taking in foraging behaviour or increased susceptibility to disease or infection for these stocks. Some indirect evidence could be interpreted in that direction, but the inferences that can be sustained are weak for the moment. For example, Myers et al. (1997) compared output from virtual population analysis with survey data and found that young fish were dying faster than could be predicted. They homed in on discarding as a potential cause, but our model suggests that the increased mortality could come from increased risk-taking in foraging behaviour or by down-regulating, e.g., immune defence. Because these fish were young and mostly immature, it is unlikely that the cause lies with maturation or reproduction.

Implications of increased mortality

In many ways, natural mortality is a loss term that removes fish from the population so that it cannot be harvested (fishermen's perspective) or its existence appreciated (conservationist's perspective). In most cases, natural mortality implies predation. The predator that benefits may be a "dead end" (jellyfish) or a marine mammal that is protected. If the model's predictions are only partly right, adaptations caused by fishing are likely to make mortality greater for the fish resources we value the most.

Quantitatively, the model's predictions were robust to parameter variation. The model predicted that natural mortality would increase by roughly half the fishing mortality. Fishing two fish would thus cause the death of a third one, once adaptations in life history traits and behaviour have taken place.

Perhaps harvest can be done differently to minimize or even reverse the evolutionary consequences (e.g., by modifying the gear's size selectivity; Law 2007; Jørgensen et al. 2009). Or perhaps one cannot harvest without causing such detrimental side effects. Regardless of the long-term consequences, a systemic increase in natural mortality necessitates increased awareness among resource managers to avoid short-term catastrophes. If an increase in natural mortality goes unnoticed in fisheries surveys, stock assessments could systematically overestimate stock size or the stock's recruitment. A dire consequence could be catch quotas that are persistently set too high with an imminent risk of stock collapse.

Earlier models

Links between life history traits and natural mortality have been considered also in earlier models, but those studies have not quantified the consequences of harvest-induced adaptations for natural mortality rates. In a model for Atlantic cod, Hutchings (2005) assumed that postreproductive mortality was higher the earlier the fish became sexually mature. He concluded that a lower maturation age is more likely to cause negative population growth, partly as a consequence of the increased postmature mortality rate. Trade-offs between natural mortality and growth, maturation, or reproduction have also been incorporated in several individual-based models for fish (e.g., Dunlop et al. 2007, 2009*a*; Enberg et al. 2009), but predictions for natural mortality rates have, to our knowledge, not been reported.

Ecosystem perspectives

In a wider ecological perspective, harvesting may have cascading effects for entire ecosystems, where not only predation rates, but also productivity and food availability, might change (Jackson et al. 2001), with potential feedback effects on traits that evolved in the first place. In a whole-lake experiment, harvesting of the prey (charr) relaxed its density-dependent regulation, with the consequence that its predator (trout) was released from stunting and subsequently recovered (Persson et al. 2007). Likewise, harvesting a predator or a prey can bring about evolutionary trait changes in the other (Gårdmark et al. 2003; Matsuda and Abrams 2004; Abrams and Matsuda 2005).

Because harvesting has removed many of the large fish predators from the world's oceans (Pauly et al. 1998), it could be argued that this would lead to reduced natural mortality on their prey. The picture is complicated by the fact that top predators other than fish have shown variable responses. For example, while some marine mammal species have declined, others are prospering and their impact as predators has increased (Clapham et al. 1999; Bowen et al. 2003). In relation to the cod example, grey seals have multiplied in the Northwest Atlantic, and although they may keep cod populations down, it seems that the reduced predation from cod on many smaller fish species is now compensated for by increased predation from seals and whales (Savenkoff et al. 2007).

How fast may species change?

The method we used, optimization, allows us to find expected evolutionary end points when little is known about heritabilities or rates of change for the manifold mechanisms that relate life history traits to mortality. How fast traits will change in natural populations depends on the type of trait. Traits that are responding directly to environmental or social cues such as behaviour often respond quickest (Lima and Dill 1990; Creel and Christianson 2008). Other traits are contingent on developmental history or are phenotypically plastic and expressed according to the experienced environment; their dynamics is slower but may still change within a life time. Examples of such traits are morphology and physiology, where alternative morphs or acclimatisation may lead to different phenotypes in different environments (Morita and Fukuwaka 2006). Finally, other traits may require genetic evolution over several generations. The rate of fishing-induced evolution has not yet been quantified directly as temporal changes in gene frequencies, so in the meanwhile, we need to rely on indirect evidence from observations on phenotypes (Dunlop et al. 2009*b*). Several forms of fishing-induced changes have taken place on decadal time scales or faster (Ricker 1981; Sharpe and Hendry 2009; see also quantification of evolutionary rates in online appendix to Jørgensen et al. 2007), but especially for observational data from the wild, there might be unknown factors and phenotypic plasticity that contribute to phenotypic trends. When accounting for known environmental trends, the rate of evolution is still fast, often taking place within decades (Grift et al. 2003; Kraak 2007; Swain et al. 2007). Other studies have suggested that evolution may be slower, either using theoretical arguments (Brown et al. 2008; Andersen and Brander 2009) or by arguing that some of the change could be explained by processes that have been omitted from the analysis (many of the arguments were presented in the theme section by Marshall and Browman 2007). Models that treat evolution explicitly suggest rates that are comparable with observed changes (Enberg et al. 2009). At present, it is difficult to conclude how fast fishing-induced evolutionary changes may be. Until alternative environmental factors are put forward that can explain the observed phenotypic trends in many fish stocks, in our view, the most parsimonious explanation is that the similar effect in widespread stocks is at least partly caused by fishing-induced adaptations.

In some cases, life history traits and behaviour are correlated and interact with the fishing activity, which can affect the strength of selection and therefore the rate of evolution. For example, angling removed a bold and fast-growing domesticated strain more rapidly than the control in a whole-lake experiment (Biro and Post 2008). In that experiment, the strongest selection took place on the first day of fishing. In addition to a direct effect on the phenotype composition in the population, it would also have a more delayed and persistent effect as the genes of the survivors are passed on to future generations.

Although many years have now passed since fishing pressure on the Canadian cod stocks was greatly reduced, the absence of fishing mortality does not necessarily imply that reverse evolution back to the original trait distributions may be fast (Law and Grey 1989). In an evolutionary individual-based model that incorporated several of the same trade-offs that we used, reverse evolution of age at maturation, growth rate, and reproductive investment was slower by a factor of 10 or more compared with the fishing-induced evolutionary change (Enberg et al. 2009). Whereas fast-reproducing strategies (early maturation, fast growth, high reproductive investment) have a great advantage when fishing mortality is high, they are nearly as good as the more slowly reproducing strategies when mortality is low, which leads to a characteristic asymmetry in evolutionary rates (Law and Grey 1989).

Predation rarely leaves an observable trace, and time series of fisheries and survey data that could have been used to detect trends over time are typically burdened by temporal variability. It is understandably tempting to focus on the more easily quantifiable measures such as size, age, and other characteristics of the fish that are alive and can be caught in fisheries or surveys. Because morphology, behaviour, physiology, life history strategies, and intra- and interspecific interactions together determine natural mortality, a cross-disciplinary effort is needed to build an understanding of the underlying mechanisms and ecological consequences. A large-scale increase in natural mortality rates induced by fishing will have consequences for fish population dynamics and fisheries productivity and raises challenges for fisheries management and policies for sustainable harvesting. Apart from the Canadian cod, a systematic increase in natural mortality has to our knowledge not been documented. Despite the difficulties in quantifying natural mortality, one would expect that an increase of the magnitude that our model predicts would not go unnoticed, as it could lead to a persistent lack of fit in population and assessment models in regular use today.

Acknowledgements

We thank Dag L. Aksnes, Katja Enberg, and Marc Mangel for comments and Doug Swain and an anonymous researcher for insightful and constructive reviews. Part of the research and writing was conducted while C.J. was visiting the Mangel lab at the University of California Santa Cruz, funded by the Research Council of Norway.

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Appendix A. Discrete time approximations of continuous time functions

The continuous time integrals were solved numerically by subdividing each year into J shorter steps. Knowing the weight at a given time $a + j/J$, weight in the next of these smaller steps, $a + (j+1)/J$, can be found as

$$w(a+(j+1)/J) = w(a+j/J) + [1-\alpha(a,l(a))]\cdot h\cdot w^{b}(a+j/J)$$

By doing this for every small time step j between a and $a + 1$, the discrete time approximation of the continuous time integral is

$$w(a+1) = w(a) + [1-\alpha(a,l(a))]\,h\int_{t=a}^{a+1} w^{b}(t)\,\mathrm{d}t \approx w(a) + [1-\alpha(a,l(a))]\,h\frac{1}{J}\sum_{j=1}^{J} w^{b}(a+j/J)$$

Gonad size at the end of the year, $g(a + 1)$, is found in an analogous way:

$$g(a+1) = \alpha(a,l(a))h\frac{1}{J}\sum_{j=1}^{J} w^{b}(a+j/J)$$

Mortality consists of four components. In the equation below, size-independent mortality is M_{fixed}. Predation mortality depends on size, which changes throughout the year so it needs to be updated and summed over the shorter time steps j. The summation is thus predation mortality $cl^{-d}(a + j/J)$, which in addition to being a separate component of mortality is multiplied by $\varphi(a, l(a))$ to get the growth-related mortality and $[q(a + j/J)/q_{\text{ref}}]^{r}$ to get the reproduction-related mortality. The final component is fishing mortality, which is specified by the harvest rate F and may include a size-selectivity function $Y(L)$ that depends on size:

$$z(a) = M_{\text{fixed}} + \frac{1}{J}\sum_{j=1}^{J}\left[cw^{-d}(a+j/J)\left(1+\varphi(a,l(a))+\left(\frac{q(a+j/J)}{q_{\text{ref}}}\right)^{r}\right)+F\cdot y(l(a+j/J))\right]$$

Having found the total mortality rate over the year ($z(a)$), annual survival probability ($s(a)$) is equal to $\exp(-z(a))$.

Contemporary and historical patterns of connectivity among populations of an inland river fish species inferred from genetics and otolith chemistry

Ryan J. Woods, Jed I. Macdonald, David A. Crook, Daniel J. Schmidt, and Jane M. Hughes

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Abstract: Patterns of dispersal in riverine populations of Australian smelt (*Retropinna semoni*) were examined using otolith chemistry (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) and genetic markers (allozymes, mtDNA, microsatellite DNA). During a period of prolonged low flows, young-of-year smelt were collected from 13 streams within three catchments of the southern Murray–Darling Basin, Australia. Spatial differences in otolith core and edge chemical signatures and high levels of genetic assignment to sampling locations were observed, suggesting that most recruits were retained in natal areas after spawning. Following a subsequent period of hydrological connection, the same cohort was sampled as 1-year-olds. Maximum likelihood estimation using otolith core chemistry data from these fish suggested that retention in natal areas was highly variable between years and a similar, though less pronounced, pattern was evident in genetic assignments. Partitioning of genetic variation among catchments was not significant ($F_{CT} < 0.004$) and probably reflects disequilibrium between migration and genetic drift due to an historical population expansion (~270 000 years ago). Taken together, otolith chemistry and genetic analyses suggest that contemporary dispersal of smelt within these catchments is relatively restricted and may be mediated by changes in hydrological connectivity.

Résumé : Des analyses de la chimie des otolithes (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) et des marqueurs génétiques (allozymes, ADNmt, ADN des microsatellites) nous ont servi à examiner les patrons de dispersion des populations de rivière de l'éperlan australien (*Retropinna semoni*). Durant une période prolongée de débits réduits, nous avons récolté des jeunes éperlans de l'année dans 13 cours d'eau dans trois bassins du réseau hydrographique sud des fleuves Murray–Darling, Australie. Nous avons observé des différences spatiales dans les signatures chimiques du noyau et de la périphérie des otolithes et de forts niveaux d'assignation génétique aux stations d'échantillonnage, ce qui indique que la plupart des recrues restent dans les zones natales après la fraie. Nous avons échantillonné la même cohorte à l'âge de 1 an après une période subséquente de connexion hydrologique. Une estimation de vraisemblance maximale faite sur les données de chimie du noyau des otolithes chez ces poissons indique que la rétention dans les aires de naissance est très variable d'une année à l'autre; il existe un patron semblable, bien que moins prononcé, dans les assignations génétiques. Le partitionnement de la variation génétique entre les bassins n'est pas significatif ($F_{CT} < 0.004$) et reflète probablement le déséquilibre entre la migration et la dérive génétique causé par une expansion de la population dans le passé (il y a ~270 000 ans). En combinaison, les analyses chimiques des otolithes et les analyses génétiques laissent croire que la dispersion actuelle des éperlans dans ces réseaux hydrographiques est relativement restreinte et peut s'expliquer par les changements dans la connectivité hydrologique.

[Traduit par la Rédaction]

Received 17 June 2009. Accepted 6 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 15 June 2010.
J21264

Paper handled by Associate Editor Eric Taylor.

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Can. J. Fish. Aquat. Sci. 67: 1098–1115 (2010) doi:10.1139/F10-043 Published by NRC Research Press

Introduction

Rivers are dendritic environments characterized by networks of hierarchically arranged habitat patches in which hydrologic and biotic connectivities are often temporally and spatially variable (Fagan 2002; Grant et al. 2007). Considering the changes to hydrology predicted to occur as a result of anthropogenic climate change and increased human demands for water resources (Miles et al. 2000; Mimikou et al. 2000; Commonwealth Scientific and Industrial Research Organisation (CSIRO) and Australian Bureau of Meteorology 2007), questions regarding the role of dispersal in the persistence of riverine organisms are of increasing importance to the conservation of aquatic biodiversity (Hughes 2007; Lassalle et al. 2008).

The behavioural and physical processes that influence connectivity for a given species operate over a range of temporal scales, from events within the lifetime of an individual (e.g., floods, droughts) to evolutionary time scales (e.g., ice ages, geological eras). A variety of methodological approaches are now available to examine biotic connectivity over these time scales (Lucas and Baras 2000). For example, analysis of the chemical constituents of fish otoliths (ear stones) has recently been used to examine within-lifetime patterns of connectivity among fish populations (e.g., Thorrold et al. 2001; Gillanders 2002; Crook and Gillanders 2006). The otoliths of fish grow continuously throughout life and are composed of a calcium carbonate matrix that is not remetabolized once deposited (Campana 1999). Dissolved trace elements in the surrounding water become chemically bonded within the otolith matrix as it accretes, and the concentrations of trace elements can thus be used to characterize the ambient water chemistry at different stages of life, including recent growth at the otolith edge and early life stages at the otolith core (Forrester and Swearer 2002; Gillanders and Joyce 2005; Sandin et al. 2005). By comparing the natal signatures of fish within a population, it is possible to estimate the recruitment sources of individuals and levels of connectivity between populations (Thorrold et al. 2001; Ruttenberg and Warner 2006; Barbee and Swearer 2007).

The use of genetic methods, in particular the analysis of variation in allozymes, mitochondrial DNA, and microsatellite DNA, is also well established in studies of population structure and dispersal in freshwater fish (Avise 2004). Various models of population structure that capture major differences in connectivity of river networks have been adopted (Hughes et al. 2009). For example, the stream hierarchy model (SHM; Meffe and Vrijenhoek 1988) predicts that the hierarchical structuring of a river network will covary with genetic structure because gene flow is more likely to occur between populations that are nested together in the river network. Where these models fail to account for observed data, other models that do not assume equilibrium between migration and genetic drift may be useful for inferring regional patterns of dispersal. These relate the degree of geographic separation among populations with their genetic differentiation (Slatkin 1993; Hutchison and Templeton 1999). In addition, dispersal estimates can be obtained using individual assignment methods that test whether an individual is a member of a particular breeding population based on the likelihood of its multilocus genotype arising by random mating in a given population of genotypes (Manel et al. 2005). Provided that sufficient genetic differentiation exists among populations, such an approach can be used to classify an individual as a "resident" or "immigrant" to its population of origin (Paetkau et al. 2004). By employing the range of available genetic techniques in such ways, analysis of population genetics can provide estimates of dispersal over time scales ranging from the lifetime of an individual fish to millennia.

Most previous studies of connectivity and structure among fish populations have used methods such as otolith chemistry and genetic techniques in isolation. More recently, however, researchers have begun to recognise the potential to increase inference by combining techniques to obtain independent estimates of population structure. Miller et al. (2005), for example, used otolith chemistry and microsatellite data to show that populations of the marine black rockfish (*Sebastes melanops*) exhibit localized spatial structuring. Similarly, identification of distinct population subdivisions was corroborated by otolith and microsatellite data in fluvial populations of Arctic grayling (*Thymallus arcticus*; Stamford and Taylor 2005; Clarke et al. 2007), Sacramento splittail (*Pogonichthys macrolepidotus*; Baerwald et al. 2007; Feyrer et al. 2007), and stocks of Chinook salmon (*Oncorhynchus tshawytscha*; Miller et al. 2010). Based on microsatellite data, limited broad-scale dispersal was inferred for northern hemisphere rainbow smelt (*Osmerus mordax*) populations, and otolith analysis confirmed that most rainbow smelt remain in estuaries during their first year of life (Bradbury et al. 2008).

Although combined analyses of otolith chemistry and genetic data often provide complementary information, in some cases there are apparent conflicts between estimates of population structure using the two methods. For example, several otolith chemistry analyses have revealed high levels of local recruitment in some marine fish, whereas genetic analysis indicated little evidence for population structure (Swearer et al. 1999 cf. Haney et al. 2007; Thorrold et al. 2001). These differences have been explained by recognising that patterns of genetic variation are contingent on a variety of processes (e.g., population size and history) in addition to the contemporary pattern of spatial connectivity that is resolved by otolith chemistry data. The chronological properties of otoliths can provide a record of movements between different aquatic environments within the lifetime of an individual, whereas genetic markers may resolve population structure over various time scales depending on the rate that variation accumulates at a given locus.

The aim of the present study was to use a suite of genetic and otolith chemistry techniques to investigate the population structure of a small fish species, the Australian smelt (*Retropinna* sp.), in small ephemeral waterways. The stochastic nature of ephemeral river systems impose spatial and temporal limits on population connectivity because opportunities for dispersal and gene flow are restricted to periods when flow is sufficient to allow movement between refugial pools. Additionally, the temporal and spatial patchiness of water in ephemeral systems renders their fish faunas highly vulnerable to the predicted impacts of climatic change, particularly in regions experiencing increased aridity. For example, hydrological connectivity may be fundamentally altered by reduced flows in the future, with likely consequences for the demography of species such as the Australian smelt. Within this context, we examine spatial variation in otolith chemistry and genetic data of Australian smelt to elucidate contemporary patterns of population structure and historical and contemporary processes relating to connectivity between hydrologically isolated populations. Specifically, we set out to determine (*i*) whether results of the two techniques indicate similar levels of contemporary dispersal in riverine populations of the Australian smelt, and (*ii*) whether Australian smelt populations are panmictic, reflecting widespread migration within the system, or fit the stream hierarchy model, with populations from the same river more similar than populations from different rivers.

Materials and methods

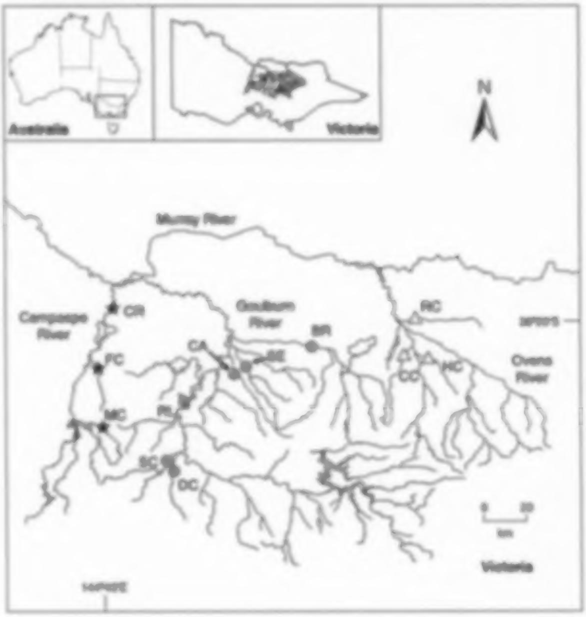
Study species

The Australian smelt is a common and abundant small-bodied (<100 mm total length) freshwater fish species complex, widely distributed throughout coastal and inland drainages in southeastern Australia. It is a member of the family Retropinnidae (southern smelts), which share a close evolutionary relationship with northern hemisphere Osmeridae (northern smelts) and southern Galaxiidae (Waters et al. 2002). Species in these groups exhibit a range of diadromous and wholly freshwater life history strategies (McDowall 1988). The Australian *Retropinna* are short-lived fish (up to 3 years), represented by two recognised species, including the widely distributed *Retropinna semoni* (Weber) in eastern Australia and *Retropinna tasmanica* (McCulloch) in Tasmania. Hammer et al. (2007) recently highlighted the need for taxonomic reappraisal of Australian *Retropinna* by demonstrating a pattern of cryptic diversity more consistent with the presence of five species. Population-level analyses indicate that *R. semoni* from the upper Murray–Darling Basin (MDB) (including the location of the present study) comprise a genetically homogenous group (MTV taxon after Hammer et al. 2007). The present analysis is therefore unlikely to be compromised by inclusion of multiple cryptic taxa, and we hereafter refer to the study species as Australian smelt or smelt. There is little detailed knowledge of the extent of movements by the species, although potamodromous migrations within freshwater have been reported in several studies of fish passing through fish ladders (e.g., Mallen-Cooper 1999; Baumgartner 2004). At least some coastal populations are thought to display facultative diadromy, with some larvae and early juveniles spending time in the estuarine or marine environment before undertaking upstream migrations into freshwater (Crook et al. 2008). There is no possibility that the smelt sampled in the current study had spent time in the marine environment, however, as the mouth of the Murray River (into which the study streams ultimately flow) is more than 1500 km downstream, with multiple dams and weirs acting as complete migration barriers along this distance.

Sampling sites

Smelt were collected from 12 ephemeral tributaries of the catchments of the Ovens, Campaspe, and Goulburn rivers in the southern MDB, southeastern Australia. A single site on the main channel of the Campaspe River was also sampled (Fig. 1). The Ovens River is one of the least flow-regulated rivers in the MDB, and although there are several small dams in the upper reaches of the catchment (total catchment area, 798 100 ha), a relatively natural flow regime is maintained throughout the year. Within the Campaspe (404 800 ha) and the larger Goulburn (1685 700 ha) catchments, substantial barriers to movement (dams and weirs) have been constructed since European settlement, and flow is heavily regulated. The tributaries that we sampled typically experience periods of low flow in the summer months, resulting in a series of hydrologically disconnected pools that act as refugial habitat for fish. Such conditions were evident in all three catchments for several months preceding sampling in February 2004 and February 2005. However, in the intervening period during winter and spring 2004 (June to November), increased flows temporarily restored hydrologic connectivity between pools in all of the study tributaries.

Fig. 1. Locations of collection sites within the Campaspe, Goulburn, and Ovens catchments. Solid stars represent sites in the Campaspe catchment (CR, Campaspe River; FC, Forest Creek; MC, McIvor Creek). Shaded circles represent sites in the Goulburn catchment (BR, Broken River; SE, Seven Creeks; CA, Castle Creek; RL, Reedy Lake Creek; SC, Sugarloaf Creek; DC, Dabyminga Creek; GL, Glen Creek). Shaded triangles represent sites in the Ovens catchment (RC, Reedy Creek; CC, Castle Creek; HC, Hurdle Creek).



Fish collection

Young-of-year (YOY) and 1-year-old smelt were collected using a 1.5 m × 7 m fine-mesh seine net in February 2004. In 2005, 1-year-old smelt (*i.e.*, the 2004 YOY cohort) were collected for otolith chemistry analysis to allow us to estimate dispersal following re-establishment of hydrological connectivity in the tributaries during spring and winter 2004. A random sample of YOY and 1-year-old individuals were used for genetic analysis in both 2004 and 2005. Upon capture, each fish was euthanized in an ice slurry and dissected to remove the head for otolith removal and the rest of the fish for genetic analysis. Each fish was given a unique identification number and the heads were stored in separate plastic bags in 95% ethanol. The body of each fish was placed in a separate plastic bag, immersed in liquid nitrogen, and subsequently transported back to the laboratory and stored at −80 °C prior to molecular analysis. Ageing of smelt was validated by annual increment ageing on the otoliths of a subset of fish and thereafter body length was used (Crook et al. 2008). Body length over 50 mm total length (TL) was considered representative of smelt 1+ years old.

Otolith preparation and analysis

The sagittal otoliths of 261 YOY fish and 141 1-year-old fish were dissected, cleaned of adhering tissue, triple-rinsed in Milli-Q water, and stored dry in polypropylene microtubes. One sagitta from each fish was then embedded whole, proximal surface facing downwards, on an acid-washed glass slide in a two-part epoxy resin (EpoFix®, Struers AS, Ballerup, Denmark). Otoliths were polished to the level of the primordium using a series of wetted lapping films (particle diameter 9, 5, and 3 μm) followed by a final polish with alumina slurry (0.5 μm). Up to 200 otoliths were mounted on each slide and arranged in random order to remove the potential for systematic measurement errors. After mounting, slides were allowed to dry and then were sonicated in Milli-Q water for 5 min and air-dried overnight in a plastic laminar flow cabinet.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was used to determine the elemental concentrations of the otoliths. For the 2004 samples, we used a Merchantek LUV 266 nm Nd:YAG UV laser microprobe (New Wave Research Inc., Fremont, California) operated in Q-switched mode and coupled to a Finnigan MAT ELEMENT high resolution ICPMS (Thermo Finnigan MAT GmbH, Bremen, Germany) located at Monash University, Melbourne, Australia. Each otolith was ablated from the distal to the proximal surface using a 30 μm diameter ablation spot (*i*) through the otolith edge at the widest radius of the dorsal margin and (*ii*) through the primordium. Data for edge and core signatures were selected from the first 15 s of the acquisition sequence for each otolith sample, which equated to ~150 laser scans. Based on examination of validated daily growth increments using similar methods to those described by Macdonald et al. (2008), we estimate that the data selected at the core incorporated the primordium and <10 days of otolith growth after hatch and that data selected at the otolith edge incorporated the last ~15 days of otolith growth prior to capture. Because of the limited dispersal opportunities available in the months preceding sampling in 2004, chemical signatures from the otolith edges (i.e., recent growth region) were considered to be representative of the environment at the collection sites. Otolith material sampled in the core regions was considered to represent the fish's natal chemical environment.

The laser was operated with output energy of 0.45–0.50 mJ and a repetition rate of 6 Hz. Ablation occurred inside a sealed chamber in an atmosphere of helium (flow rate, 0.36 L·min^{-1}) with the ablated materials mixed with argon prior to transport to the ICPMS. Otoliths were analysed for several isotopes, including ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba. ^{43}Ca was measured for use as an internal standard to correct for variation in ablation yield among samples. The concentrations of the other isotopes were standardized to ^{43}Ca using the relative response of the instrument to known elemental concentrations in an external glass standard (National Institute of Standards Technology, NIST 612), and data were expressed as analyte to Ca molar ratios (i.e., Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca). The ablation chamber was purged for 20 s after each opening to remove any background gas or particles from previous samples. Mean counts of a 20 s background analysed prior to each ablation transect were subtracted from the averaged raw ion counts for each element over the interval of interest. The NIST 612 was analysed after every 10 samples to correct for any long-term drift in the instrument. Blank backgrounds, which measured the sample gases without ablation, were measured before each sampling session and used to determine detection limits. Detection limits (in μg·g^{-1}) were calculated based on the ablation yield equivalent to 3 × standard deviation (SD) of the blank signal. Mean values for each element were as follows: Mg, 0.73; Mn, 0.08; Sr, 0.09; and Ba, 0.01. Precision of the method was calculated using the percentage of relative standard deviation (%RSD) of mean concentrations of elements ablated from 150 laser scans of the NIST 612. Averaged values across all NIST 612 samples (n = 37) were as follows: Mg:Ca, 3.1%; Mn:Ca, 4.2%; Sr:Ca, 2.4%; and Ba:Ca, 3.2%.

In 2005, otolith chemistry analysis was conducted using a Resonetics LPX120i ArF 193 nm excimer laser system (Resonetics, Nashua, New Hampshire) coupled to an Agilent 7500s ICPMS (Agilent Technologies, Inc., Santa Clara, California) at the Australian National University (ANU). The uniform energy distribution and rapid response of this laser system allowed us to more precisely isolate data from the growth region of interest than the 266 nm laser system used to analyse the cores and edges of the 2004 samples (see Macdonald et al. 2008). We used a depth-profiling approach to sample a 70 × 6 μm target region at the otolith core that incorporated 2–10 days of otolith growth after hatch, but excluded material associated with the primordium (identified by a clear peak in Mn concentrations). The otolith core regions of the 141 1-year-old smelt collected in February 2005 were analysed in this way. To remove potential errors associated with interlaboratory comparisons (Campana et al. 1997), we also re-analysed the core regions of the second sagitta from 238 of the YOY smelt collected in February 2004 using the ANU system. The laser system was calibrated using NIST 610 and NIST 612 reference standards, and measurements of the concentrations of ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba were acquired for each otolith sample. Average detection limits (3 × SD of the blank signal (in μg·g^{-1})) for each element across all samples were as follows: Mg, 0.091; Mn, 0.042; Sr, 0.009; and Ba, 0.011. Precision values (%RSD) based on 22 means of a sequence of 39 laser scans (i.e., the same number of pulses used for otolith samples) on the NIST 612 were as follows: Mg:Ca, 8.22%; Mn:Ca, 7.81%; Sr:Ca, 7.58%; and Ba:Ca, 10.35%. Further information on the analytical procedures and laser operating parameters used can be found in Macdonald et al. (2008), and for a detailed description of the laser system's performance, see Eggins and Shelley (2002) and Eggins et al. (1998, 2005).

Analysis of otolith chemistry data

Spatial patterns in otolith edge and core chemistry for the 2004 samples were examined with univariate and multivariate ANOVAs and linear discriminant function analysis (DFA) using data from the Monash University system. Classification success for the DFAs was calculated by jackknifed cross-validation matrices, and standardized coefficients for the discriminant functions were used to measure which elements contributed most to site separation. We conducted randomization tests to determine if the jackknifed classification estimates were significantly different from random (see White and Ruttenberg 2007). Based on code provided by White and Ruttenberg (2007), a script was run in R (version 2.8.0, The R Foundation for Statistical Computing) to calculate the classification success rates and associated *P* values (i.e., the probability of obtaining the observed classification rate due to chance alone) using uniform prior probabilities and 10 000 randomizations of the data. All analyses were performed on $\log_e$-transformed data.

Maximum likelihood estimation (MLE) was used to estimate the proportion of the 1-year-old fish captured in 2005 that had remained at their natal site since the previous summer. We used the 2004 YOY otolith core chemical signatures for each sampling site acquired using the ANU system (n = 238) as the baseline data set. Univariate and multivariate analyses of variance (ANOVAs) were conducted to ensure that the results were consistent between the Monash and ANU systems. Otolith chemistry data for the Broken River site was not considered suitable as baseline data because of the wide spread in the multi-elemental chemical signatures of fish from this site. Although exclusion of this site reduces the spatial extent of our analysis, our emphasis was to estimate the proportion of 1-year-old fish assigned as residents within each site, rather than attempting to describe all potential sources of fish. Thus, removal of the Broken River site does not affect the overall conclusions drawn from the otolith chemistry analysis. The MLEs were conducted for each catchment separately using the software program HISEA (Millar 1990), and we used the direct maximum likelihood estimator for all calculations. To investigate the variability of the estimator, 1000 simulations (with resampling) were run on the baseline data set (Campaspe, n = 59; Goulburn, n = 99; Ovens, n = 59). Following the simulations, the software was run in analysis mode and the proportion of putative resident 1-year-old fish at each site was estimated.

Allozymes

Preparation of muscle tissue for allozyme electrophoresis followed methods in Hughes and Hillyer (2006). Initially, three individuals from each population were screened for polymorphism across 25 enzyme systems based on previously published protocols (Richardson et al. 1986). Five polymorphic loci (frequency of common allele < 0.99) were detected and genotyped for 30 individuals from each population using two standard individuals run on each plate. Locus names, abbreviations, and enzyme commission codes include glucose-6-phosphate isomerase (*PGI*, 5.3.1.9), aspartate transaminase (2 loci, *AAT*-1 and *AAT*-2, 2.6.1.1), β-esterase (*βEST*, 3.1.1.1), and peptidase B (*PEP-B*, 3.4.1.1).

Microsatellites

Total genomic DNA was extracted from muscle tissue following a cetyltrimethyl ammonium bromide (CTAB) – phenol – chloroform extraction protocol (Doyle and Doyle 1987). The 30 individuals used for allozyme analysis from each population were genotyped for four polymorphic microsatellite loci (*Sm18*, *Sm26*, *Sm49*, and *Sm80*) (Hillyer et al. 2006). PCR reactions and product preparation for electrophoresis followed the methods described by Hillyer et al. (2006). Individuals were genotyped at each locus using a 5% denaturing acrylamide gel on a Gel-Scan 2000 DNA analyser (Corbett Research, Sydney, Australia). Allele size was determined against a Genemark 350-TAMRA size marker (Astral Scientific, Gymea, NSW, Australia) along with two internal PCR standards on each gel. Alleles were visualized and scored using ONE-DSCAN software (Scanalytics Inc., Fairfax, Virginia).

Mitochondrial DNA

A region of the mtDNA genome spanning ATPase 6 and ATPase 8 was amplified according to the polymerase chain reaction (PCR) protocol of McGlashan and Hughes (2000). Amplified product from five individuals was sequenced in both directions, and a new reverse primer was developed and used in further amplifications, smelt r-7 (5′-AGCCGTAGGTTGGTTGGTTTCGCAT-3′). Amplified product was purified via an enzymatic purification protocol using exonuclease 1 (Fermentas International Inc., Burlington, Ontario, Canada) and shrimp alkaline phosphatase (Promega Corporation, Madison, Wisconsin) and sequenced on an ABI 3130xl (Applied Biosystems, Life Technologies Corporation, Carlsbad, California) automated sequencer at the Griffith University DNA Sequencing Facility. Twenty individuals were sequenced from each population; these were randomly selected from the 30 individuals used in allozyme and microsatellite analysis. Haplotype sequences were lodged with GenBank under accession nos. FJ593511 to FJ593561 (Appendix A, Table A1).

Intrapopulation diversity

Tests for linkage disequilibrium and departures in genotypic proportions expected from Hardy–Weinberg equilibrium (HWE) were calculated with exact tests for each locus–population combination using default settings in GENEPOP (Raymond and Rousset 1995). Population-level diversity was estimated for allozyme and microsatellite markers using expected heterozygosity and allelic richness in GENEPOP. Mitochondrial sequences were edited and aligned using BIOEDIT (Hall 1999). Genetic variation within populations was assessed using haplotype diversity (*h*) in ARLEQUIN (version 3.1; Excoffier et al. 2005). Nucleotide variation across the study area was used to test for historical changes in the demography of smelt in the upper Murray–Darling Basin. Dramatic changes to population size (e.g., bottlenecks and population expansions) may alter both haplotype diversity and variation in segregating sites relative to the patterns expected for stable populations (Ramos-

Onsins and Rozas 2002). Fu's F_S statistic (Fu 1997) was used to test for a signature of population expansion that manifests in an excess of haplotypes relative to expectations for a historically stable population. The R_2 statistic (Ramos-Onsins and Rozas 2002) was used to test for an excess of singleton substitutions (i.e., base changes sampled only once), which are likewise characteristic of rapidly expanding populations. In addition, a mismatch distribution of pairwise base differences among all individuals was calculated for comparison with the distribution expected for an exponentially growing population. An estimate of the time of the expansion was taken using the expectation $\tau = 2ut$ (Rogers 1995), where τ is the age of the expansion in mutational units derived from the mismatch distribution, u is the per-generation nucleotide substitution rate for the gene fragment based on a yearly per-nucleotide substitution rate of 0.65×10^{-8} (Bermingham et al. 1997) and average generation time of one year (Pusey et al. 2004), and t is the unknown expansion time in generations. Fu's F_S, the mismatch distribution, and R_2 were calculated in DnaSP (version 4.50; Rozas et al. 2003).

Population genetic structure and individual assignment

Pairwise comparisons of genetic structure were calculated for each genetic marker using F_{ST} estimates in ARLEQUIN (version 3.1; Excoffier et al. 2005). The critical value (α) was corrected for multiple tests using the BY false discovery rate method (BY-FDR), which controls experiment-wide type I error without the loss of power to detect differentiated populations, that is associated with the Bonferroni adjustment (Benjamini and Yekutieli 2001; Narum 2006). Mantel tests (Mantel 1967) were used to test for isolation-by-distance (IBD) where Slatkin's linearized F_{ST} (Slatkin 1995) was used as a measure of genetic differentiation, and river distances (Fetzner and Crandall 2003) were measured from a topographic map with a scale of 1 : 250 000. Tests for IBD were conducted across all sample sites separately for mtDNA, microsatellites, and allozymes markers.

The influence of hierarchical river structure on spatial genetic variation was tested with analysis of molecular variance (AMOVA) in ARLEQUIN (version 3.1; Excoffier et al. 2005) for each genetic marker. The hierarchical levels of analysis and their associated F statistics include among catchments (F_{CT}), among sites within catchments (F_{SC}), and among all sites (F_{ST}). Sequence divergence was incorporated into the AMOVA for the mtDNA data by calculating Φ_{ST} based on uncorrected pairwise differences between haplotypes.

Individuals were classified as either residents or first-generation (F_0) immigrants to populations represented by 30 individuals collected at each sampling location in 2004, using individual assignment and population exclusion methods implemented in GENECLASS (version 2; Piry et al. 2004). Individual assignment probabilities were calculated using the partial Bayesian approach of Rannala and Mountain (1997) using four microsatellite loci. The test statistic used for classification of each individual as resident or immigrant was the likelihood of drawing the individual's genotype from its population of origin (L_h), which is appropriate to the present situation in which all potential source populations are not sampled (Paetkau et al. 2004). The simulation algorithm of Paetkau et al. (2004) was used to generate 10 000 resident genotypes and derive an associated distribution of assignment probabilities for each location. Individuals were excluded as residents and classed as immigrants when L_h fell within the 0.01 tail of this distribution (Paetkau et al. 2004). Re-establishment of hydrological connectivity during spring and winter 2004 allowed us to examine whether the potential for dispersal had a detectable impact on individual assignment rates for smelt collected in 2005. Eleven sites were successfully resampled in 2005, and approximately 20 individuals from each site were assigned to reference data collected from the same location in 2004 before hydrological connection occurred.

Results

Spatial variation in otolith chemical signatures

Substantial differences in the concentrations of Mn:Ca, Sr:Ca, and Ba:Ca in otolith edges were observed both among and within catchments, yet Mg:Ca concentrations were relatively similar across all sites based on data from the Monash laser system (Table 1*a*; Fig. 2). The results of the multivariate ANOVAs and linear DFAs showed that it was possible to discriminate between most sampling sites within catchments using the multi-elemental signatures of the otolith edges (Table 1*b*; Fig. 3). There was substantial overlap in the otolith edge chemical signatures of fish from the different catchments, resulting in only moderate classification accuracy across catchments (61%, $P < 0.001$) (Table 2). However, sites within catchments tended to be more distinct, with overall classification success rates of 67% ($P = 0.032$) in the Campaspe, 83% (<0.001) in the Goulburn, and 97% ($P < 0.001$) in the Ovens catchments. The lower classification success among the Campaspe sites was mainly due to the similarity in otolith edge signatures between Forest Creek and McIvor Creek smelt (Table 2; Fig. 2). Examination of the standardized coefficients showed that Mn:Ca was most influential in the discriminant functions at the catchment scale but had little influence at the site scale. Sr:Ca and, to a lesser extent, Ba:Ca were responsible for much of the observed variation in otolith edge chemistry among sites (Table 3).

Similar to the otolith edges, the analysis of core otolith chemistry revealed significant univariate and multivariate differences in elemental concentrations among catchments and among sites within catchments, with comparable results obtained using both the Monash and ANU laser systems (Table 1; Figs. 2, 3). Because of the lack of hydrological connectivity at most sites prior to sampling, YOY smelt collected in February 2004 are likely to have been restricted to their natal site since they were spawned. Hence, we would expect relatively high classification success of individuals based on otolith core chemistry. The linear DFAs based on data from the Monash system confirm this and show clear discrimination among most sampling sites within catchments, with classification accuracy similar to and, in many cases, higher than those of the otolith edges (Table 2; Fig. 3). Ba:Ca and Mn:Ca were most influential in the discriminant analysis at the catchment scale. At the site scale, Sr:Ca and Ba:Ca were most important, whereas Mn:Ca had relatively little influence (Table 3).

Table 1. (*a*) Mean squares (MS) and significance levels for a two-way nested analysis of variance (ANOVA) on mean log$_e$-transformed Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca in the edge and core regions of young-of-year (YOY) Australian smelt otoliths (n = 261) analysed on the Merchantek LUV 266 nm laser at Monash University (Monash) and in the core region of the second otolith from 238 of these fish analysed on the Resonetics 193 nm laser at the Australian National University (ANU). (*b*) Results from one-way multivariate ANOVAs on log$_e$-transformed Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca in the edge and core regions of YOY Australian smelt otoliths analysed at Monash University (n = 261) and in the core region of the second otolith from 238 of these fish analysed at the ANU. Comparisons are made among the three river catchments (Campaspe, Goulburn, Ovens) and among sites within catchments.

(*a*) Results from two-way nested analysis of variance (ANOVA)

		MS Mg:Ca		MS Mn:Ca		MS Sr:Ca		MS Ba:Ca	
Source	df	Edge	Core	Edge	Core	Edge	Core	Edge	Core
Monash									
Catchment	2	0.165	0.154	14.087***	13.581***	0.749***	0.608***	9.172***	11.489***
Site (catchment)	9	0.272	0.243	3.831***	2.326***	1.675***	1.907***	5.956***	2.815***
Error	249	0.231	0.177	0.238	0.313	0.020	0.016	0.077	0.053
ANU									
Catchment	2		0.002		7.595***		0.297***		10.882***
Site (catchment)	9		0.027*		1.089***		1.227***		1.447***
Error	226		0.010		0.299		0.015		0.064

(*b*) Results from one-way multivariate ANOVAs

		Pillai's trace		*F*	
Source	Source, error df	Edge	Core	Edge	Core
Monash					
Catchment	8, 512	0.375	0.520	14.757***	24.286***
Sites (Campaspe)	8, 124	0.489	0.780	5.022***	10.071***
Sites (Goulburn)	20, 492	2.063	1.766	26.213***	19.442***
Sites (Ovens)	8, 120	1.714	1.564	89.792***	53.831***
ANU					
Catchment	8, 446		0.602		25.104***
Sites (Campaspe)	8, 108		0.842		9.809***
Sites (Goulburn)	20, 456		1.489		13.517***
Sites (Ovens)	8, 108		1.253		22.648***

Note: The 12 sampling sites from which YOY fish were collected are nested within the three river catchments (Campaspe, Goulburn, Ovens). df, degrees of freedom; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

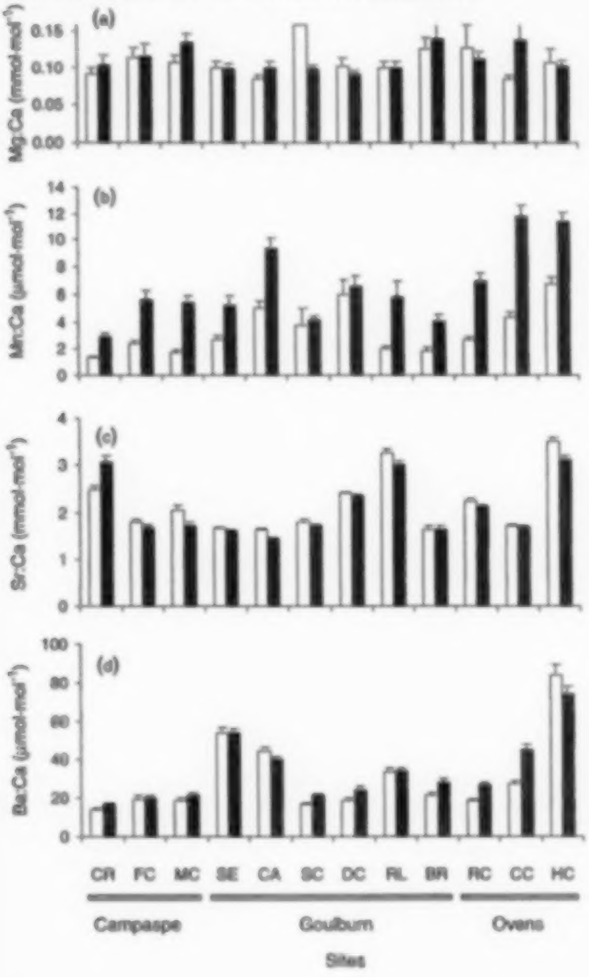
Dispersal estimation using otolith core chemistry

Estimates of the proportions of 1-year-old fish classified as residents or immigrants were undertaken using the otolith core chemistry data acquired with the ANU system. We were unable to collect any 1-year-old fish at several sites, whereas at others (e.g., Croppers Creek, Hurdle Creek), interpretation was limited by low sample sizes (Table 4). In the Campaspe catchment, 95% (n = 21) of 1-year-old smelt collected from McIvor Creek were assigned to their site of collection (i.e., putative residents) based on their otolith core chemistry, and all 27 fish collected from the Campaspe River were classified as residents (Table 4*a*). By contrast, estimates for the Goulburn catchment suggested that at most sites, a high proportion of 1-year-old fish were nonresidents that had migrated into the sampling site since February 2004. Of the 55 1-year-old smelt analysed, only 16% were classified as residents based on their otolith core chemistry. None of the fish collected at Castle Creek (n = 9) or Seven Creeks (n = 10) was assigned to their collection sites, and 47% (n = 23) of fish collected from Reedy Lake Creek were classified as residents (Table 4*b*). In the Ovens catchment, it was estimated that 94% (n = 17) of 1-year-old smelt captured at Reedy Creek were residents (Table 4*c*). The low numbers of 1-year-old smelt captured at Croppers Creek and Hurdle Creek limits interpretation for these sites, although 88% (n = 8) of fish collected from Hurdle Creek and Croppers Creek were classified as nonresidents.

Intrapopulation genetic diversity

Genetic diversity was higher for microsatellite loci than for allozymes in all populations, with microsatellite allelic richness and heterozygosity ranging from 6.25 to 11.25 and from 51% to 80%, respectively, compared with the lower allozyme values ranging from 2.0 to 3.0 and from 20% to 42% (Table 5). Tests for HWE and linkage disequilibrium indicate no evidence for systematic deviations from random assortment at any locus in both 2004 and 2005 samples. A 502 bp alignment of ATPase from 260 individuals (20 individuals/population) included 46 variable positions and 51 unique haplotypes. Moderately high haplotype diversity was observed in all populations, with the number of haplotypes sampled in each population ranging from 4 to 13 (Table 5; Appendix A, Table A1).

Fig. 2. Mean (+ standard error, SE) concentrations of (*a*) Mg:Ca, (*b*) Mn:Ca, (*c*) Sr:Ca, and (*d*) Ba:Ca in the edge (open bars) and core (solid bars) regions of Australian smelt (*Retropinna semoni*) otoliths collected from 12 sites in the Campaspe, Goulburn, and Ovens River catchments. Otolith analyses were conducted on the Merchantek LUV 266 nm laser system operated at Monash University. Sites: CR, Campaspe River; FC, Forest Creek; MC, McIvor Creek; SE, Seven Creeks; CA, Castle Creek; SC, Sugarloaf Creek; DC, Dabyminga Creek; RL, Reedy Lake Creek; BR, Broken River; RC, Reedy Creek; CC, Castle Creek; HC, Hurdle Creek.



Statistical tests for population growth based on the mtDNA locus were consistent with a historical population expansion event. The observed Fu's F_S value of –56.5 ($P < 0.001$) is consistent with an excess of haplotypes relative to expectations for a stable population. The small R_2 value of 0.022 ($P < 0.001$) reflects an excess of singleton substitutions in the mtDNA data set, which is consistent with a historical growth event. The shape of the mismatch distribution is unimodal, smooth, and left-skewed as expected for a population that has undergone a historically recent expansion. Assuming that population growth is responsible for this pattern of variation, then the timing of the expansion event (τ = 1.788) is on the order of 275 000 years.

Spatial structuring of genetic variation

Structuring of genetic variation was nonrandom across populations for mtDNA (Φ_{ST} = 0.213), allozymes (F_{ST} = 0.095), and microsatellites (F_{ST} = 0.045), indicating that smelt are not panmictic in the study area (Table 6). Pairwise comparisons indicated that a large proportion of populations were significantly differentiated from each other. The BY-FDR corrected critical value of $P \le 0.00987$ was used for 78 pairwise comparisons and 40 (51%) tests were significant for mtDNA, whereas 60 (77%) and 51 (65%) tests were significant for microsatellites and allozymes, respectively. Hierarchical structuring of the river network in accordance with the SHM was a poor predictor of genetic structure. Partitioning of variation among the catchments (F_{CT}) is expected to be relatively high according to the SHM, but we found the reverse, with catchments accounting for less than 0.3% of variation in a hierarchical AMOVA and the corresponding F_{CT} values are low and nonsignificant (Table 6). This result was consistent across each of the three genetic marker types used and for most loci except *Sm18* and *PGI* (Table 6). Current barriers to movement in the regulated rivers do not appear to explain the observed pattern of genetic structuring. For example, significant structuring in the Campaspe catchment is attributable to differentiation between upper (McIvor Creek) and lower (Forest Creek, Campaspe River) populations rather than between the two latter populations, which are separated by the Campaspe Weir.

Tests for isolation by river distance across the study area were nonsignificant for each of the three genetic markers (mtDNA, r = –0.106, P = 0.6 (Fig. 4*a*); allozymes, r = –0.067, P = 0.64 (Fig. 4*b*); microsatellites, r = –0.179, P = 0.8 (Fig. 4*c*)). Three populations are responsible for a disproportionate number of high F_{ST} values in the IBD scatterplots (Fig. 4). For the mtDNA data, all 19 pairwise comparisons with $F_{ST} > 0.3$ involve Sugarloaf Creek and Dabyminga Creek samples. These two populations, along with McIvor Creek, are responsible for 20 of the 24 pairwise $F_{ST} > 0.06$ for the microsatellite data. For the allozyme data set, McIvor Creek alone accounts for 10 of the 14 comparisons of $F_{ST} > 0.15$.

Dispersal estimation using genetics: classification of resident–immigrant status

The null hypothesis of resident status for fish collected in 2004 was rejected for 13 out of a total of 390 individuals (3.3%) from 13 locations throughout the study area. In any given population, ≥93% of individuals fell within the expected distribution of assignment scores for resident genotypes (Table 4). Immigrant genotypes were distributed across nine populations, including all catchments. Exclusion tests for each of the 13 putative immigrants against all populations in the same catchment revealed that five "immigrants" were excluded as residents from all sampled populations and the remaining eight "immigrants" could not be excluded as residents from multiple populations. It therefore appears likely that some of the genetically identified immigrants represent true F_0 immigrants whose source

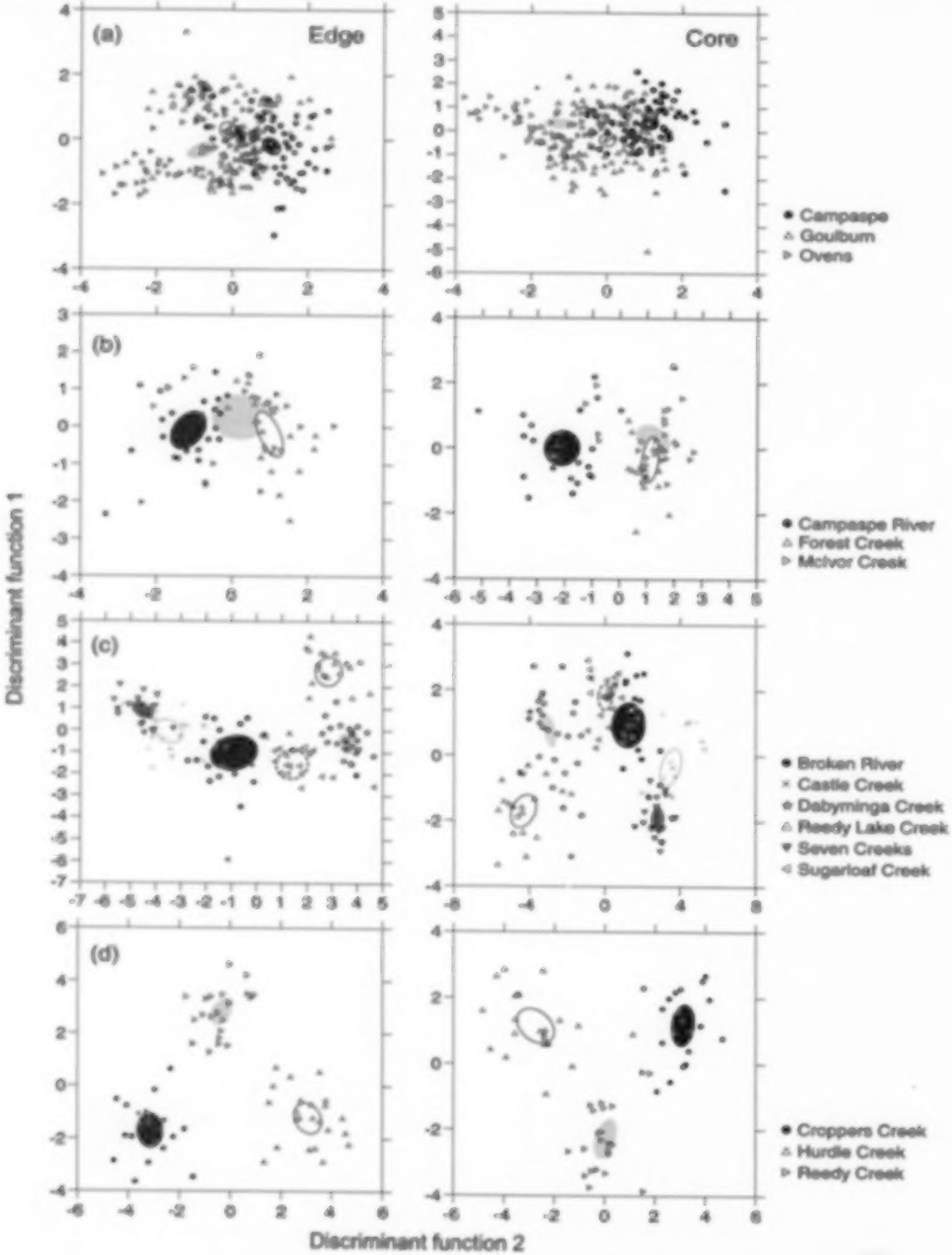


Fig. 3. Discriminant function plots displaying spatial variation in multi-elemental signatures (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) of edge and core regions of young-of-year (YOY) Australian smelt (*Retropinna semoni*) otoliths collected in February 2004: (*a*) among catchments and among sites within (*b*) the Campaspe, (*c*), the Goulburn and (*d*) the Ovens catchments. Ellipses represent 95% confidence intervals around the group centroid. Data points represent multi-elemental otolith signatures for individual smelt, and plots are based on $\log_e$-transformed data from the Monash University laser system.

Table 2. Success of classifying individual young-of-year (YOY) Australian smelt collected in February 2004 to their collection site based on the otolith chemistry of the edge and core regions (*a*) among catchments and within (*b*) the Campaspe catchment, (*c*) the Goulburn catchment, and (*d*) the Ovens catchment.

		Otolith edge		Otolith core	
	n	% correct	*P*	% correct	*P*
(*a*) Among catchments					
Campaspe	67	75		78	
Goulburn	129	58		54	
Ovens	65	54		72	
Total	261	61	<0.001	65	<0.001
(*b*) Within the Campaspe catchment					
Campaspe River	23	83		91	
Forest Creek	21	86		71	
McIvor Creek	23	35		57	
Total	67	67	0.032	73	<0.001
(*c*) Within the Goulburn catchment					
Broken River	21	81		67	
Castle Creek	21	71		86	
Dabyminga Creek	26	88		92	
Reedy Lake Creek	19	95		95	
Seven Creeks	19	84		84	
Sugarloaf Creek	23	78		96	
Total	129	83	<0.001	87	<0.001
(*d*) Within the Ovens catchment					
Croppers Creek	20	90		100	
Hurdle Creek	23	100		96	
Reedy Creek	22	100		95	
Total	65	97	<0.001	97	<0.001

Note: *P* values refer to the probability of obtaining the observed total classification success rate due to chance only. Analyses were based on data from the Monash University laser system.

Table 3. Standardised coefficients and percent dispersion explained for the first two discriminant functions (DF1, DF2) in analyses of intercatchment and within-catchment otolith (*a*) edge and (*b*) core chemical signatures of young-of-year (YOY) Australian smelt collected in February 2004.

	Intercatchment		Campaspe		Goulburn		Ovens	
	DF1	DF2	DF1	DF2	DF1	DF2	DF1	DF2
(a) Edge								
Mg:Ca	0.210	0.022	0.156	0.346	0.054	0.331	0.115	0.362
Mn:Ca	–0.668	0.061	0.297	–0.927	0.351	–0.324	0.143	–0.162
Sr:Ca	–0.114	1.057	–0.802	–0.063	1.145	0.576	1.135	0.879
Ba:Ca	–0.471	–0.555	0.395	0.752	–1.146	0.603	–0.230	–1.364
Dispersion (%)	78.4	21.6	95.4	4.6	76.9	15.4	68.1	31.9
(b) Core								
Mg:Ca	0.003	0.175	0.160	0.935	0.176	0.189	0.160	0.361
Mn:Ca	–0.443	0.623	0.158	–0.163	0.147	0.004	0.227	0.187
Sr:Ca	–0.174	0.776	–0.918	0.227	–1.026	–0.286	–1.107	–0.387
Ba:Ca	–0.768	–0.565	0.329	0.497	0.511	–0.899	0.191	1.144
Dispersion (%)	84.7	15.3	97.4	2.6	78.9	18	68.2	31.8

Note: Analyses were based on data from the Monash University laser system.

population was not sampled in the present study, whereas others may represent type I errors (true residents that are incorrectly rejected within the tolerated threshold of $\alpha = 0.01$ used for these tests). Eleven sites were resampled in 2005 after hydrological connection occurred during spring and winter 2004. The null hypothesis that each individual collected in 2005 belonged to the population of genotypes sampled from the same location in 2004 was rejected for 12

Table 4. Assignment tests of resident status for individuals in each population based on multilocus genetic data for 2003 and 2004 cohorts (Genetics 2004) and 2004 and 2005 cohorts (Genetics 2005).

	Genetics 2004		Genetics 2005		Otolith chemistry	
Site	*n*	% resident	*n*	% resident	*n*	% resident
(a) Campaspe catchment						
Campaspe River	30	93	20	95	27	100
Forest Creek	30	100	0	—	0	—
McIvor Creek	30	97	20	100	21	93
Total or average	90	97	40	98	48	97
(b) Goulburn catchment						
Broken River	30	100	20	100	0	—
Castle Creek	30	93	19	100	9	0
Dabyminga Creek	30	97	20	90	0	—
Reedy Lake Creek	30	93	20	100	23	47
Seven Creeks	30	97	20	75	10	0
Sugarloaf Creek	30	97	20	95	13	17
Glen Creek	30	100	0	—	0	—
Total or average	210	97	119	93	68	16
(c) Ovens catchment						
Croppers Creek	30	97	20	100	2	50
Hurdle Creek	30	100	20	85	6	0
Reedy Creek	30	93	20	100	17	94
Total or average	90	97	60	95	25	72

Note: Maximum likelihood estimation (MLE) using otolith core chemistry data for the 2004 cohort collected as 1-year-olds in February 2005 (otolith chemistry). Otolith analyses were conducted separately for each of the three catchments ((*a*) Campaspe, (*b*) Goulburn, and (*c*) Ovens) and were based on data from the ANU laser system. Assignments using otolith chemistry methods were conducted on 1-year-old fish, and genetic assignments included young-of-year (YOY) and 1-year-old fish. Total or average: total *n* values, average % resident.

out of a total of 219 individuals (5.5%). The proportion of individuals from each 2005 site that fell within the expected distribution of assignment scores for 2004 ranged from 75% to 100% (Table 4).

Discussion

Contemporary dispersal

Analysis of the otolith chemistry and genetic data provide important insights into the within-lifetime dispersal histories of Australian smelt. Analysis of the genetic data from the 2004 samples, which included both YOY (2004 cohort) and 1-year-old fish (2003 cohort), showed that the most pronounced structuring of variation in all markers occurred between relatively closely spaced populations within catchments. Most relevant to contemporary dispersal patterns, the assignment tests based on microsatellite variation provide information on whether the genetic composition of an individual is the result of random mating within a population of resident parental genes or if it is an immigrant whose genetic makeup is derived from a different population of parental genes. The low number (<4%) of immigrants detected using these tests provides evidence that the genetic variation recovered from each sampling location is representative of the existence of a series of stationary local populations and suggests very limited dispersal by the 2003 and 2004 cohorts prior to their collection in summer 2004.

Analysis of the otolith chemistry data for the YOY fish collected in summer 2004 (2004 cohort) yields a similar interpretation, indicating that genetics and otoliths yield complimentary information on population structure of smelt. Given the distinctive otolith chemical signatures of YOY fish observed among sites within catchments, as evidenced by the analysis of otolith edge chemistry, disparate natal (core) chemical signatures would have been expected among fish from particular sites if recruits had dispersed throughout or among catchments from one or more common spawning grounds. However, the tightly grouped and spatially distinct natal chemical signatures of fish from the vast majority of sites (the Broken River was an exception) strongly suggest that most new recruits were retained in localized areas within catchments after spawning. An alternative explanation for this pattern is that recruits dispersed throughout or among catchments in nonmixing groups with distinctive core chemistry signatures that were not reflective of the sites from which the fish were sampled. The likelihood that such an explanation has a significant effect on the interpretation of our results appears remote, however, considering the similarity in spatial patterns between the core and edge chemical signatures and the lack of hydrological connectivity prior to sampling. In the more ephemeral streams (e.g., McIvor Creek, Forest Creek, Castle Creek), the finding of limited dispersal by the 2004 cohort is not surprising because dispersal of juvenile fish away from their natal site was unlikely to be possible for several months prior to sampling due to a lack of hydrological connectivity caused by extended cease-to-flow conditions (R.J. Woods and D.A. Crook, personal observations). However, flow occurred dur-

Table 5. Location of sampling sites and genetic diversity statistics for each population sampled in 2004 (2003 and 2004 cohorts) and 2005 (2004 and 2005 cohorts).

		2004									2005		
		Allozymes			Microsatellites			mtDNA			Microsatellites		
Sampling site	Location	*n*	Ar	H_e	*n*	Ar	H_e	*n*	n_{hap}	*h*	*n*	Ar	H_e
Campaspe catchment													
Campaspe River	36°15′S, 144°44′E	30	2.8	0.26	30	10.75	0.71	20	9	0.87±0.04	20	8.83	0.87
Forest Creek	36°39′S, 144°33′E	30	3.0	0.32	30	9.50	0.77	20	5	0.56±0.11	—	—	—
McIvor Creek	36°56′S, 144°43′E	30	2.4	0.29	30	9.25	0.74	20	5	0.66±0.09	20	7.18	0.66
Goulburn catchment													
Broken River	36°28′S, 145°53′E	30	2.0	0.22	30	10.25	0.77	20	10	0.91±0.03	20	6.51	0.80
Castle Creek	36°37′S, 145°22′E	30	2.6	0.28	30	11.25	0.76	20	8	0.69±0.10	19	6.73	0.77
Dabyminga Creek	37°06′S, 145°07′E	30	2.0	0.20	30	6.25	0.67	20	4	0.55±0.11	20	6.63	0.81
Reedy Lake Creek	36°44′S, 145°07′E	30	2.4	0.28	30	11.00	0.78	20	10	0.86±0.05	20	6.48	0.82
Seven Creeks	36°36′S, 145°29′E	30	2.6	0.26	30	8.50	0.72	20	5	0.51±0.12	20	7.85	0.82
Sugarloaf Creek	37°04′S, 145°03′E	30	2.2	0.36	30	6.25	0.51	20	5	0.66±0.09	20	4.75	0.61
Glen Creek	36°19′S, 145°20′E	30	2.4	0.42	30	10.25	0.80	20	9	0.84±0.06	—	—	—
Ovens catchment													
Croppers Creek	36°27′S, 146°22′E	30	2.4	0.26	30	11.00	0.76	20	10	0.71±0.11	20	7.31	0.81
Hurdle Creek	36°31′S, 146°25′E	30	2.4	0.27	30	10.75	0.79	20	13	0.91±0.05	20	8.29	0.87
Reedy Creek	36°20′S, 146°21′E	30	2.8	0.26	30	10.75	0.76	20	10	0.83±0.07	20	6.94	0.75

Note: Ar, allelic richness; H_e, expected heterozygosity; n_{hap}, number of unique haplotypes sampled within a population; *h*, haplotype diversity.

Table 6. Analysis of molecular variance to test for hierarchical structuring of genetic variation in the 2004 sample.

Locus	Among catchments (F_{CT})	Among populations within catchments (F_{SC})	Among all populations (F_{ST})
Multiple loci			
Microsatellites (four loci combined)	0.002	0.043***	0.045***
Allozymes (five loci combined)	0.003	0.093***	0.095***
Individual loci			
mtDNA	0.000	0.236***	0.213***
Sm49	0.000	0.041***	0.037***
Sm18	0.014*	0.043***	0.056***
Sm26	0.007	0.034***	0.041***
Sm80	0.000	0.055***	0.044***
PGI	0.041*	0.045***	0.085***
AAT-1	0.000	0.011***	0.006
AAT-2	0.000	0.039*	0.031*
βEST	0.000	0.055***	0.047***
PEP-B	0.000	0.143***	0.127***

Note: Significance determined by 10 000 random permutations of sampling units among predefined hierarchical groupings (i.e., populations among catchments (F_{CT}), individuals among populations within catchments (F_{SC}), and individuals among populations (F_{ST}). *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.

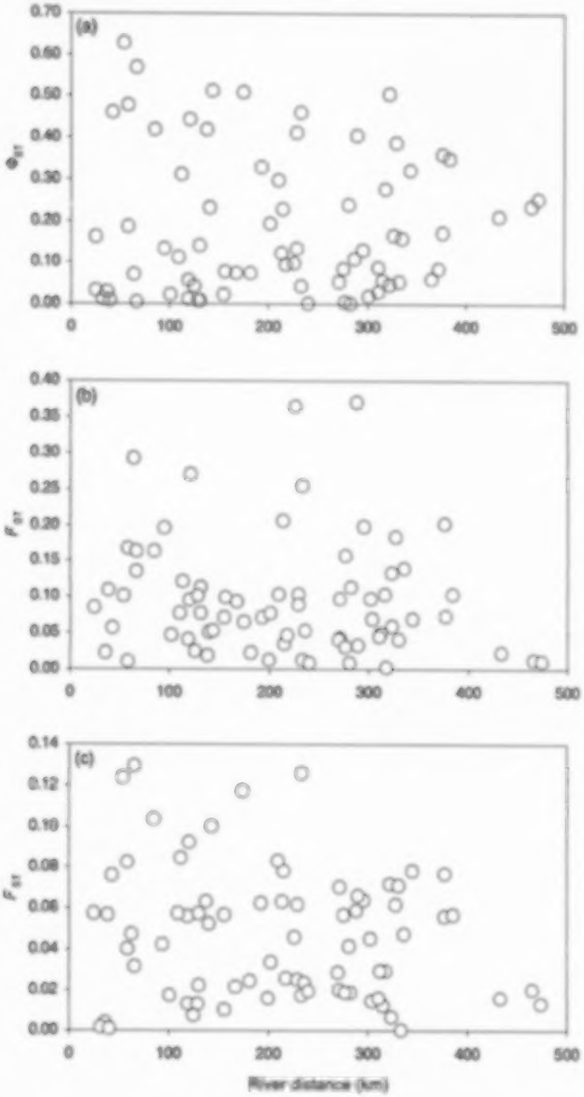
ing or immediately prior to sampling at some sites (e.g., Campaspe River), and at least some dispersal of juveniles may have been possible but apparently did not occur to a large extent at most sites.

Sampling of the 2004 cohort of fish as 1-year-olds in 2005 for otolith chemistry analysis was conducted following a period in which all of the study sites had experienced periods of hydrological connection during winter and spring. Unlike the genetic and otolith chemistry results for the 2004 samples, the core otolith chemistry assignment tests suggested that retention of 1-year-old individuals was highly variable among sites in 2005. Given the similarities in otolith core chemistry of YOY fish collected in 2004 at some sites across the different catchments, some potential exists for our retention estimates to be biased by intercatchment immigration during the periods of hydrologic connection. However, the large geographic distance among the catchments, the presence of numerous instream barriers, and the relatively short duration of reconnection during winter and spring 2004 suggest that such large-scale movements are unlikely to strongly influence our findings. Overall, the otolith chemistry results suggest that dispersal of smelt may be both spatially and temporally variable and that hydrologic connectivity is likely to be a major factor in determining the rates of dispersal within catchments. Likewise, the genetic analysis of 2005 fish showed greater among-site variation in assignment success relative to the 2004 sample. It is notable that otolith chemistry indicated high retention of 1-year-old individuals in the Campaspe and Ovens catchments but more variation in the Goulburn catchment. A similar pattern was reflected in the genetic assignment results. Populations in the Goulburn catchment had consistently high rates of genetic assignment success in 2004 (ranging from 93% to 100%), with more variation evident in 2005 (75%–100%). Although otolith chemistry and genetic analysis of smelt were each able to detect similar patterns of spatial population structure in 2004, the response to connectivity in 2005 was more noticeable in the otolith analysis. The difference in resolving power of the two methods might be explained by more pronounced variation in water chemistry over small spatial scales relative to variation in population allele frequencies. An increase in power to correctly detect immigrant individuals using the genetic approach would be achievable with the use of more microsatellite loci (Paetkau et al. 2004).

Historical dispersal

Analysis of otolith and genetic data at the level of individual populations indicates limited contemporary dispersal of smelt during periods of low hydrological connectivity and spatially variable dispersal following higher flows that reinstate connectivity. Although the otolith chemistry results suggest that immigrants moved into many of the study sites between the summer of 2004 and the summer of 2005, this provides no information on the distance moved by individuals, and thus, we are unable to determine the spatial extent of contemporary connectivity between local populations from the otolith chemistry data. Nevertheless, if the present-day dispersal is spatially restricted and this is indicative of a long-term historical pattern, we might expect partitioning of genetic variation among the three catchments to be relatively large and significantly nonrandom in accordance with the stream hierarchy model (Meffe and Vrijenhoek 1988). Similarly, if equilibrium between limited migration and genetic drift were established throughout the study region, then a positive correlation would be expected between population differentiation and river distance (Slatkin 1993; Hutchison and Templeton 1999). As neither of these models was supported by the genetic data, we infer that (*i*) long-term equilibrium between drift and migration is not established and (or) (*ii*) that the observed spatial pattern of differentiation is the product of stochastic sorting of genetic variation into the refugial pools that form during dry periods of the year. The latter hypothesis (*ii*) is analogous to

Fig. 4. Isolation-by-distance scatterplots showing relationship between genetic differentiation and geographic distance for all pairwise combinations of 13 population samples collected in 2004: (*a*) mtDNA data set; (*b*) allozyme data set; and (*c*) microsatellite data set.



“patchy recruitment” (sensu Bunn and Hughes 1997) or “family sampling” (sensu Allendorf and Phelps 1981) whereby a sample of individuals is representative of a limited number of families rather than the wider population. In this way, colonization of refugial pools by closely related cohorts of juvenile smelt at the end of spring and early summer might result in the population-level differentiation observed at small spatial scales. This scenario might be supported by extensive deviations from Hardy–Weinberg proportions across loci and sampling sites due to nonrandom mating (Bunn and Hughes 1997; Hughes et al. 1998). However only a small number of these deviations were observed in the present study, as is typically the case in similar studies of freshwater fish populations (e.g., Huey et al. 2008).

The hypothesis of nonequilibrium between migration and genetic drift across the study area (i.e., hypothesis *i*, above) is supported by the observed relationship between river distance and genetic differentiation, which resembles the hypothetical outcome expected when a homogenous source population invades a region, followed by dramatic attenuation of among-population gene flow (Hutchison and Templeton 1999). According to this scenario (i.e., case III, Hutchison and Templeton 1999), the scatter of points in the IBD plot results from stochastic sorting of genetic variation into populations due to rapid colonization of a region, followed by small-scale fragmentation of populations (“microvicariance,” Templeton et al. 1990), which increases variance in the differentiation between pairs of populations without regard to the geographic distance separating them. Nonsignificant IBD patterns similar to those observed in this study are occasionally observed in studies of river fish taxa (e.g., Castric et al. 2001; Costello et al. 2003). In these cases, a historically recent colonization of the river system is usually high on the list of suspected biological mechanisms to account for the pattern. Nucleotide variation at the mtDNA locus is compatible with the nonequilibrium hypothesis as this satisfies a model of rapid expansion in population size across the study area occurring during the late Pleistocene. Although it seems clear that a balance between genetic drift and migration has not been reached across the study area, further study is required to establish whether patchy recruitment is contributing to the observed patterns of genetic variation. Analysis of genetic variation over multiple years within sites would be useful to address this question because temporal differentiation would be expected to match or exceed spatial variation if refugial pools are colonized under a model of patchy recruitment (Hughes et al. 1998).

Implications for river management

It appears that despite evidence of some localized movement, contemporary among-population movement of Australian smelt within the three study catchments is restricted to at least some extent and that this has resulted in genetic structuring among local populations. This finding is particularly interesting in light of previous studies in larger, more perennial lowland rivers that have reported large numbers of Australian smelt undertaking potamodromous migrations through fishways (Mallen-Cooper et al. 1995). Such apparently disparate behaviour by smelt in different environments suggests that connectivity between local and regional populations of this species differs between small ephemeral waterways and larger lowland rivers. This has clear implications for the management of rivers and their fisheries, as it suggests that specialized strategies may be required to conserve the genetic and ecological integrity of populations of a single species depending on the environmental (e.g., hydrological) characteristics of the ecosystem under consideration.

Integrating methods of otolith chemistry and genetic analysis, as demonstrated here, provides a promising means of interpreting the contemporary responses of species to changes in land and water management practices within the context of their premanagement history. Given the imminent threats to aquatic biota in ephemeral waterways posed by factors such as predicted climate change and increasing human demands for water, an understanding of contemporary and historical movement patterns of species at the landscape scale is critical to the future development of strategies to conserve biodiversity in aquatic ecosystems.

Acknowledgements

We thank Damien O'Mahony for help in collecting samples and David Gopurenko and Mark Ponniah for assistance in the lab. Funding was provided by eWater CRC, CRC for Freshwater Ecology, Australian Rivers Institute, Griffith University School of Environmental Science, and an Australian Postgraduate Award (R.J.W.). Procedures were carried out in accordance with Australian Ethics Approval No. AES 03/04/AEC.

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Appendix A

Table A1. mtDNA haplotype codes and their sample frequency at each site.

	GenBank no.	CR	FC	MC	BR	CA	DC	RL	SE	SC	GL	CC	HC	RE
Hap1	FJ593511	5	13	3	1	11	4	5	14		4	11	6	8
Hap2	FJ593512	5		1	4	3		2	2		1	1	1	3
Hap3	FJ593513			1		1								
Hap4	FJ593514	2	1	11			13		1					
Hap5	FJ593515			4										
Hap6	FJ593516	3	3		2			1		3			3	2
Hap7	FJ593517		1											
Hap8	FJ593518		2								1			
Hap9	FJ593519	1												
Hap10	FJ593520	1			1	1								
Hap11	FJ593521	1												
Hap12	FJ593522	1												
Hap13	FJ593523	1												
Hap14	FJ593524				2	1								1
Hap15	FJ593525				4	1	2	6			7	1		1
Hap16	FJ593526				3									
Hap17	FJ593527				1									
Hap18	FJ593528				1									
Hap19	FJ593529				1								1	
Hap20	FJ593530								1					
Hap21	FJ593531								2					
Hap22	FJ593532					1								
Hap23	FJ593533					1		1		4	1			1
Hap24	FJ593534									11				
Hap25	FJ593535									1				
Hap26	FJ593536									1				

Table A1 (*concluded*).

	GenBank no.	CR	FC	MC	BR	CA	DC	RL	SE	SC	GL	CC	HC	RE
Hap27	FJ593537						1							
Hap28	FJ593538							1						
Hap29	FJ593539							1						
Hap30	FJ593540							1						
Hap31	FJ593541							1				1	1	
Hap32	FJ593542							1						
Hap33	FJ593543										1	1		1
Hap34	FJ593544										3			
Hap35	FJ593545										1			
Hap36	FJ593546										1			
Hap37	FJ593547													1
Hap38	FJ593548												1	1
Hap39	FJ593549													1
Hap40	FJ593550											1		
Hap41	FJ593551											1		
Hap42	FJ593552											1		
Hap43	FJ593553											1		
Hap44	FJ593554											1		
Hap45	FJ593555												1	
Hap46	FJ593556												1	
Hap47	FJ593557												1	
Hap48	FJ593558												1	
Hap49	FJ593559												1	
Hap50	FJ593560												1	
Hap51	FJ593561												1	
Total		20	20	20	20	20	20	20	20	20	20	20	20	20

Note: Site codes: CR, Campaspe River; FC, Forest Creek; MC, McIvor Creek; BR, Broken River; CA, Castle Creek; DC, Dabyminga Creek; RL, Reedy Lake Creek; SE, Seven Creeks; SC, Sugarloaf Creek; GL, Glen Creek; CC, Castle Creek; HC, Hurdle Creek; RC, Reedy Creek.

Sensitivity of pH in a boreal stream network to a potential decrease in base cations caused by forest harvest

Anneli Ågren, Ishi Buffam, Kevin Bishop, and Hjalmar Laudon

Abstract: Increased forest harvest with more whole-tree utilization can decrease base cations (BC) in soils and stream runoff. This paper analyses how reducing stream BC changes the capacity of a boreal stream network to buffer pH changes. We estimated change in stream pH during spring snowmelt in 60 locations throughout a 68 km^2 boreal catchment in northern Sweden with different scenarios of BC removal from stream water ranging from 10 to 50 μequiv.·L^{-1}. The pH decreased in all scenarios, and if BC decreased by 50 μequiv.·L^{-1}, stream length with pH above the acid threshold pH 5 during spring snowmelt decreased from 82% to 44% of the stream network, whereas the stream length with pH above 5.5 decreased from 60% to 10%. The pH sensitivity of different stream reaches to reductions in BC was positively related to the slope of the catchment, forest cover, and forested mires, whereas it was negatively related to the percentage of agricultural fields. Because the long-term effect of different forestry practices on stream BC is unclear, there is all the more reason to evaluate BC sensitivity before, rather than after, eventual problems arise.

Résumé : La récolte accrue des forêts avec l'utilisation élargie des arbres entiers peut réduire les cations basiques (BC) dans les sols et l'écoulement des cours d'eau. Notre étude analyse comment la réduction des BC dans le cours d'eau modifie la capacité d'un réseau d'eau courante boréal à tamponner les changements de pH. Nous avons estimé les changements de pH dans les cours d'eau durant la fonte des neiges au printemps à 60 points dans un bassin versant boréal de 68 km^2 dans le nord de la Suède avec divers scénarios de retrait des BC des eaux courantes allant de 10 à 50 μequiv.·L^{-1}. Le pH a diminué dans tous les scénarios; lorsque les BC avaient diminué de 50 μequiv.·L^{-1}, la longueur de cours d'eau ayant un pH au-dessus du seuil acide de pH 5 durant la fonte des neige était réduite de 82 % à 44 % du réseau, alors que la longueur de cours d'eau avec un pH au-dessus de 5,5 avait baissé de 60 % à 10 %. La sensibilité au pH des diverses sections de cours d'eau en fonction de la réduction des BC est en corrélation positive avec la pente du bassin versant, la couverture de forêt et les tourbières forestières, alors qu'elle est en corrélation négative avec le pourcentage de champs agricoles. Parce que les effets à long terme des différentes stratégies forestières sur les BC des cours d'eau restent imprécis, c'est une raison de plus d'évaluer la sensibilité des BC avant, plutôt qu'après, l'arrivée éventuelle de problèmes.

[Traduit par la Rédaction]

Introduction

There is increasing interest in forest biomass harvest in many regions of the world. Plans to increase productivity and use new extraction methods, including whole-tree harvesting (WTH), are being put into action to meet an increasing demand for biofuel (European Commission 1997) and other forest products. In WTH, the whole aboveground biomass is extracted (in Sweden, the practice of removing the stump wood and roots has been implemented on a trial basis on 1% of the final cut area). The impacts of such forest management decisions on ecosystem services such as future soil productivity and water quality is a topic of current interest. One particular concern is how increased biomass removal from the catchment soils will affect the long-term export of base cations (BC, defined as the sum of Ca, Mg, Na, and K) to surface waters in acid-sensitive regions.

Soils and surface waters are closely linked by groundwater, which transports dissolved nutrients and other solutes from the terrestrial to the aquatic ecosystem. A consequence of this strong linkage is that a change in the soil biogeochemistry will ultimately affect stream water quality. As the BC concentration strongly controls acid-buffering capacity in streams, a decline in catchment soil BC concentration due to biomass removal will impact the pH in the stream network.

Forest harvesting has different effects on BC over different time scales. In the short term, BC often increase in surface waters following harvest (Neal et al. 1992) due to

Received 4 September 2009. Accepted 30 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 15 June 2010.
J21384

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doi:10.1139/F10-052

Published by NRC Research Press

increased mineralization, decomposition, and possibly leaching during nitrification (Kreutzweiser et al. 2008). In a longer time perspective, which is the focus of this study, harvesting of trees will remove BC from the watershed soils and eventually contribute to surface-water acidification (Neal et al. 1992). The environmental effect on surface waters of biomass removal, through loss of soil BC, will ultimately be determined by how sensitive the soils are to BC depletion. In Mediterranean forests, MAGIC (Model of Acidification of Groundwater In Catchments; Cosby et al. 1985, 2001) models predict little change in water quality due to forestry harvest, because there are high BC inputs from the atmosphere and the BC weathering rates are also high (Neal et al. 1995). However, in the more acid-sensitive soils in regions of the northeastern US and Canada, as well as in Scandinavia, models predict soil and surface-water acidification with different forestry scenarios (Larssen 2005; Clair et al. 2007; Aherne et al. 2008). Mass-balance calculations have demonstrated that Finnish soils will be depleted in base cations in the long term if WTH is practiced (Joki-Heiskala et al. 2003). Measurements from long-term experiments (15–16 years after harvesting) also report that WTH results in lower pools of exchangeable BC, as well as a decreased cation exchange capacity in the soil compared with stem-only harvesting (Olsson et al. 1996). Another survey of the same sites 25 years after harvesting showed that the effect of WTH on the pools of exchangeable BC persisted (Olsson et al. 2004). An investigation from Canada showed that reduction of BC in the soil would also reduce the export of BC in streamwater, in particular Ca (Molot and Dillon 2008). The effects of a change in forestry practice from stem-only to whole-tree harvesting on Finnish lakes were modeled using MAGIC (Aherne et al. 2008). The authors found that the long-term (by 2100) potential decrease in surface-water BC due to WTH was, on average, 10 μequiv.·L^{-1}. In a Swedish application of MAGIC, WTH was found to cause long-term acidification of soils after one rotation period (on average, 98 years (79–141)), especially in areas with high stand productivity and low weathering capacity (Westling and Kronnäs 2006). Modeled deforestation was also predicted to decrease acid-neutralizing capacity (ANC) in runoff (1960–2060), with the decline ranging from about 20 μequiv.·L^{-1} to almost 100 μequiv.·L^{-1} depending on forest type and previous acidification history. Even without WTH, mass-balance calculations suggest that serious depletion of BC will occur after one to two rotations across large parts of Sweden (Akselsson et al. 2007).

Because of the limited understanding of how intensified forestry practice will affect the long-term sustainability of water quality at the landscape scale, there is a need for studies exploring potential impacts on aquatic ecosystems. The purpose of this study is to investigate the sensitivity of a boreal stream network to declines in streamwater BC ranging from 10 μequiv.·L^{-1} to 50 μequiv.·L^{-1}. We apply a well-tested charge-balance model for pH (Köhler et al. 2000; Hruška et al. 2003) on 60 stream segments during spring snowmelt in the Krycklan catchment network in northern Sweden (Laudon and Buffam 2008). Using this model, we examine how the potential removal of BC from the stream water (in the absence of other water chemistry changes) would affect the pH drop during spring snowmelt and how this would change the amount of suitable habitat for acid-sensitive biota in the stream network. Whereas other studies have focused on ANC, this paper focuses on pH, which is typically the key factor for acid-sensitive biota and can be used as an integrated benchmark of acid–base status in the watershed as a whole. The response in pH is analyzed in a landscape perspective, with empirical measurements of water chemistry providing the foundation.

Specifically, we ask the following. (*i*) In a mesoscale boreal catchment, given a uniform change in stream BC on the order of that expected from WTH, how much of the stream habitat will cross ecologically relevant pH thresholds? (*ii*) What are the characteristics of the stream reaches and subcatchments most sensitive to changes in BC?

Material and methods

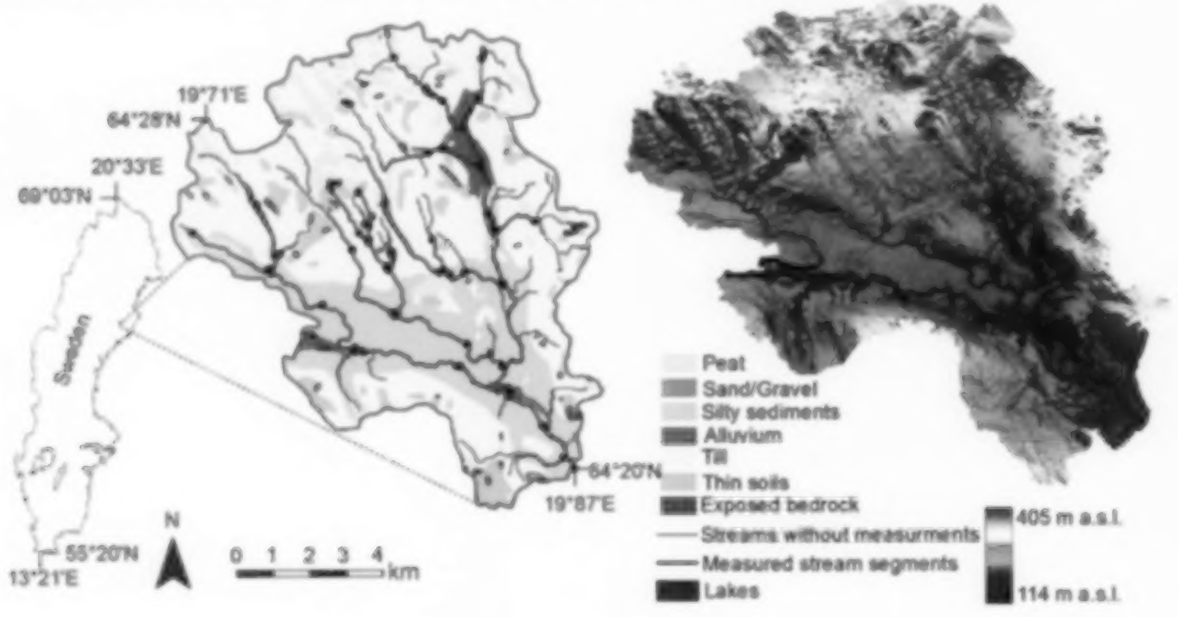
Study site

The Krycklan catchment (Fig. 1), a 68 km^2 area boreal stream network in northern Sweden, has been used as the model catchment for this study. The bedrock consists of Precambrian gneiss with intrusions of acid and intermediate metavolcanic rocks, granite, and pegmatite. The Quaternary deposits are dominated by till, with patches of peat, bare rock, thin soils, and fine sorted sediments. The Krycklan catchment ranges in elevation from 114 to 405 m above sea level (a.s.l.). In the lower part of the catchment, the streams traverse a postglacial delta, with thick layers of silty sediments where ravines and bluffs of up to 20 m can be found. In the till soils, higher up, well-developed iron podzols dominate the forest floor soils, but near the stream channels, the organic content increases, forming a riparian peat zone along the streams. The peatlands (covering 9% of the catchment) are dominated by *Sphagnum* species. These can be categorized as acid, oligotrophic, and minerogenic, with varying proportions of microtopographic units (e.g., hollows, hummocks, strings, lawns) (Rydin et al. 1999). Forests cover 84% of the catchment, clear-cuts, 4%, open or arable grounds, 3%, and lakes, 1%. The dominant tree species is Scots pine (*Pinus sylvestris*) in dry upslope areas, whereas Norway spruce (*Picea abies*) dominates in wetter, lower-lying areas. The Vindeln Experimental Forest covers 25% of the catchment area and has been protected since 1922, whereas the majority of the other 75% of the area is second-growth forest. The population in the catchment is less than 100 people. The mean annual air temperature is 1 °C, and the mean annual precipitation in the region amounts to 600 mm, of which approximately 50% becomes runoff. The area has on average five months of snow cover, and 35%–40% of the precipitation falls as snow (Eriksson 1991). The hydrology is characterized by a strong runoff peak in late April – early May due to snowmelt, with occasional rainfall-induced runoff peaks in summer and autumn. The streams are relatively humic (brown water) with dissolved organic carbon (DOC) levels ranging from 4 to 41 mg·L^{-1} (Buffam et al. 2007).

Sampling

The stream segments used in this study were defined by Buffam and co-workers (Buffam et al. 2008). In short, stream chemistry was sampled at 60 locations in the stream

Fig. 1. The location of the Krycklan catchment with the Quaternary deposits map (1 : 100 000, Geological Survey of Sweden, Uppsala, Sweden) and the topography according to a digital elevation model based on LIDAR measurements (note that the topography map is tilted for a three-dimensional effect and that height is exaggerated five times for visualization purposes). The solid circles are the sampling points. The bolder blue lines indicate the measured stream segments, and the thinner lines indicate stream segments without measurements.



network, and the chemistry was then assigned to segments of the streams to be able to express the changes in proportion to stream length. The segments were constructed by extending to the halfway point between neighboring sampling sites. At the headwater sites, segments were extended halfway to the stream origin on the 1 : 100 000 map. The mean length of the segments was 941 m (68–2874 m). Those segments represented 56 km out of a total of 96 km in the whole network. We express the changes in stream length with chemistry as a percentage of the measured stream length (56 km). Based on results from studies on fish (Holmgren and Buffam 2005; Serrano et al. 2008) and stream invertebrate species (Fölster et al. 2007), pH 5.5 and pH 5.0 were used as thresholds in this study as these were found to be the most critical pH intervals for many acid-sensitive species.

The stream network was sampled at 60 sites during the rising limb, close to peak runoff during the spring snowmelt (22 April 2004) and concurrent with the minimum pH (Buffam et al. 2007). Samples for dissolved inorganic carbon (DIC) analysis were injected into N_2-filled 60 mL glass vials and acidified. For all other analyses, stream-water grab samples were collected in 250 mL high-density polyethylene bottles. All samples were stored in the dark and at cool temperatures until subsampling for chemical analysis. The samples were analyzed for major cations (K^+, Na^+, Mg^{2+}, Ca^{2+}), strong acid anions (SO_4^{2-}, Cl^-, NO_3^-), pH, DOC (after filtration with 0.45 µm MCE membrane filter), and DIC (using partial pressure of headspace CO_2). For details regarding the analytical procedures, refer to Buffam et al. (2008) and Laudon and Buffam (2008).

pH model

A charge-balance model that predicts pH in the stream segments was constructed based on the following equations (Laudon and Buffam 2008). Assuming that K^+, Na^+, Mg^{2+}, and Ca^{2+} were present in their free ionized form, the BC concentration was calculated as

$$(1)\qquad \mathrm{BC} = [K^+] + [Na^+] + 2[Mg^{2+}] + 2[Ca^{2+}]$$

The strong acid anions (SAA) were calculated as

$$(2)\qquad \mathrm{SAA} = 2[SO_4^{2-}] + [Cl^-] + [NO_3^-]$$

BC and SAA were expressed as μequiv.$\cdot L^{-1}$ of charge. The acid-neutralizing capacity (ANC) was then calculated as BC – SAA. Another way to express ANC is

$$(3)\qquad \mathrm{ANC} = [HCO_3^-] + 2[CO_3^{2-}] + [RCOO^-] + [OH^-] - [H^+] - n[Al^{n+}]$$

where DIC is used to calculate HCO_3^-, using Henry's law and carbonate equilibria equations. CO_3^{2-} did not affect the ANC in this case, as pH was always below 7.0 and CO_3^{2-} could therefore be excluded. $RCOO^-$ denotes dissociated organic acid anions calculated from DOC and pH using a three pK_a model following Hruška et al. (2003). Al^{n+} denotes positively charged inorganic monomeric Al species. Previous studies in these and similar high DOC streams have shown that inclusion of the monomeric labile Al fraction and Fe does not significantly improve the agreement between measured and model-predicted pH at the pH range of these stream water samples (Köhler et al. 2000, 2001; Laudon et al. 2000). Because inclusion of Al^{n+} does not improve the model for these waters (Köhler et al. 2000), it was excluded. Hence, H^+ can be calculated as

$$(4)\quad [H^+] = [HCO_3^-] + [RCOO^-] + [OH^-] - BC + SAA$$

No model calibration was carried out in this study, but because HCO_3^-, $RCOO^-$, and OH^- are dependent on pH, this equation is solved iteratively, until pH was stable within 0.01 pH units.

BC scenarios

First we ran the model with present conditions at each of the 60 stream sites, and the modeled pH was compared with measured pH to assess the success of the model. Then we ran different scenarios in which ANC was changed (by changing BC). By keeping all other water chemistry constant, we studied the net effect of the BC removal on pH. In the scenarios, we removed 10, 20, 30, 40, and 50 μequiv.·L^{-1} of BC from present day BC ($BC_{present}$). The BC scenarios represent upper and lower bounds of possible effects of whole-tree harvest taken from the literature. Of course, it is unrealistic that the distribution of BC removal would be equal through the entire catchment system, but it is done to illustrate the point that different landscape units display different sensitivity. The range 0–50 μequiv.·L^{-1} corresponds to 0%–22% of the mean spring BC stream concentration in our study catchment. The key to our modeling approach is a charge-balance – H^+ buffering model, where we assume that any change in BC is offset by a change in H^+ and then allow the model to come to equilibrium given the other dissolved constituents present. Thus we use H^+ (not pH) for the modeling, but we use pH in the paper for descriptive and visual purposes because that is a more conventional way to show the changes at an ecologically relevant scale. To minimize the uncertainty of the modeling, the new pH was calculated from the measured $[H^+]$ minus the modeled change in $[H^+]$ (Laudon and Buffam 2008), as shown in eqs. 5–6. In this way, much of the uncertainty in the organic acid modeling is cancelled out.

$$(5)\quad [H^+{}_{\Delta BC}] = [HCO_3^-] + [RCOO^-] + [OH^-] - (BC_{present} - \Delta BC) + SAA$$

$$(6)\quad \Delta H^+ = H^+{}_{\Delta BC} - H^+{}_{\text{modeled at present conditions}}$$

$$(7)\quad pH_{new} = -\log(H^+{}_{measured} + \Delta H^+)$$

It should be noted that the approach we used allows pH to be estimated at discrete points; the approach does not provide explicit spatial connectivity between observation points, i.e., mixing downstream. However, the sampling design is such that different sites can be taken to represent stream segments of varying length, as in Laudon and Buffam (2008).

To answer the question "What are the characteristics of the stream reaches and subcatchments most sensitive to changes in BC?" we performed a partial least squares analysis (PLS) using the SIMCA-P 11.0.0.0 statistical package (Umetrics AB, Umeå, Sweden). The PLS model was constructed with ΔpH from three simulations as *Y* variables and 27 landscape characteristics (derived from maps and a digital elevation model) on soil types, land cover, stream order, catchment area, slope of the catchment, average height of the catchment and tree volume from satellite data (by the *k* nearest-neighbor method; Reese et al. 2003) as *X* variables. Before construction of the model, the variables were transformed to fit normality and were converted to *z* scores. For this paper, the purpose of the PLS modeling was not to predict ΔpH, but rather to determine which catchment–stream variables are associated with high ΔpH. When refining the model, an emphasis was therefore placed on reducing the number of variables down to the significant variables (95% confidence interval) with high weight.

Results

During present conditions (measured values), the BC concentration in the 60 stream segments during the spring snowmelt of 2004 was 229 (±54) μequiv.·L^{-1} (mean ± standard deviation (SD)). The base cations showed a normal distribution, checked with a one-sample Kolmogorov–Smirnov test in SPSS Statistics 17.0 (SPSS Inc., Chicago, Illinois). The average pH was 5.49 (Table 1). The comparison of modeled and measured pH suggests that the model succeeded well in predicting the pH in the stream segments during spring snowmelt (Fig. 2). The root mean square error (RMSE) between measured and modeled values was 0.15 pH units.

To put the modeled pH changes in perspective, we compared them with the observed episodic pH drop during snowmelt. From the winter baseflow to the spring snowmelt, the average pH drop was 0.87 pH units (SD = 0.37 pH units) (Fig. 3). The model predicts a further decline in pH during spring snowmelt for the different BC removal scenarios. A decrease of 10 μequiv.·L^{-1} BC would lower the pH during spring snowmelt by another 0.10 pH units on average. A decrease of 30 μequiv.·L^{-1} and 50 μequiv.·L^{-1} BC would decrease pH by another 0.31 (Fig. 3) and 0.56 pH units on average, respectively.

The decrease in pH was not uniform among streams. The largest pH effect for a given decrease in BC was found in stream segments with a contemporary snowmelt pH between 5 and 6, where the average pH decline due to a decrease in BC of 30 μequiv.·L^{-1} was 0.37 pH units. For stream segments with pH below 5 or above 6, the decrease was less pronounced, and the average decline was 0.26 and 0.22 pH units, respectively. There was a large variation in the sensitivity of the stream segments, but the PLS model (R^2X = 0.39, R^2Y = 0.32, Q^2 = 0.26) indicated that subcatchments with a steep slope and high forest cover (and also forested wetlands) were the catchments that had stream pH most sensitive to a change in BC (Fig. 4). On the other hand, the subcatchments with open fields were less sensitive in terms of ΔpH.

At present, 82% of the stream length in the catchment had a pH above 5 during spring snowmelt. The stream segments with pH below the thresholds 5 and 5.5 presently are located in the upper central part of the catchment (Fig. 5). In the BC removal scenarios, the pH changed significantly. If BC were to decrease by 30 μequiv.·L^{-1} throughout the network, the proportion of stream length with a pH above 5 would decrease to 62% (Fig. 6). A similar change was found for the

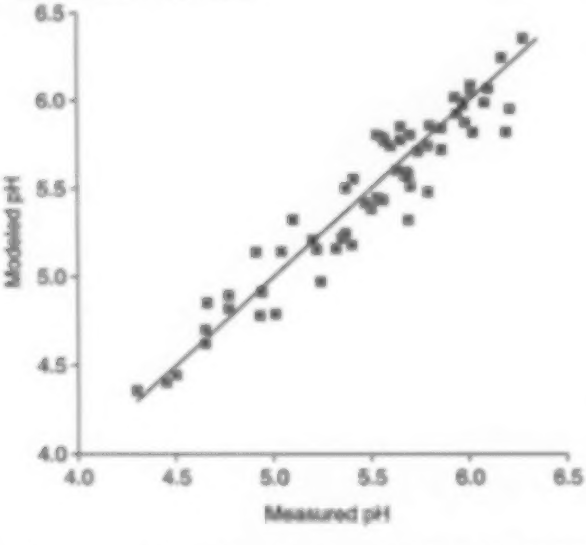
Table 1. Mean (standard deviation (SD)) of stream chemistry in the 60 stream segments during spring snowmelt of 2004.

Analyte	Mean (SD)
H^+ (μequiv.·L^{-1})	6 (9)
Ca^{2+} (μequiv.·L^{-1})	103 (31)
K^+ (μequiv.·L^{-1})	20 (11)
Mg^{2+} (μequiv.·L^{-1})	56 (12)
Na^+ (μequiv.·L^{-1})	49 (12)
SO_4^{-} (μequiv.·L^{-1})	81 (31)
NO_3^{-} (μequiv.·L^{-1})	4 (5)
Cl^- (μequiv.·L^{-1})	25 (6)
$RCOO^-$ (μequiv.·L^{-1})	118 (15)
HCO_3^{-} (μequiv.·L^{-1})	31 (27)
BC (μequiv.·L^{-1})	229 (54)
SAA (μequiv.·L^{-1})	106 (33)
ANC* (μequiv.·L^{-1})	123 (33)
DIC (μmol·L^{-1})	306 (68)
DOC (mg·L^{-1})	18 (4)
pH (pH units)	5.49 (0.49)

Note: BC, base cations; SAA, strong acid anions; ANC, acid-neutralizing capacity; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon.

*ANC was calculated as BC – SAA.

Fig. 2. Modeled pH versus measured pH for the 60 stream segments. The 1:1 line is shown.



other pH threshold, where the stream length above pH 5.5 was 60% at present and would decrease to 35% with a BC drop of 30 μequiv.·L^{-1}. If BC were to decrease by 50 μequiv.·L^{-1}, the proportion of stream length with pH above the acid thresholds pH 5 and pH 5.5 would be 44% and 10%, respectively (Fig. 6). According to the most severe scenario, with a BC removal of 50 μequiv.·L^{-1}, the majority of the segments with a pH remaining above thresholds (marked in light green on the map in Fig. 5) would be located in the silty postglacial delta area and a few reaches would be found in areas with glaciofluvial sediments (Figs. 1 and 5).

Discussion

Impacts of harvest on watershed base cations

This section gives an overview of the impacts of harvest on watershed base cations based on a literature review. The watershed BC net-flux equation can be viewed as

$$(8)\quad \text{leaching} = \text{deposition} - \text{uptake} + \text{weathering} \pm \text{change in soil adsorbed pool}$$

Tree uptake of BC is balanced by the concurrent release of H^+, maintaining the charge balance in both tree roots and soil. Hence, forests acidify soils by trees taking up BC and releasing H^+; therefore, removal of the trees will result in a decreased BC uptake, which may increase BC leaching to streams in the short term. However, the large pool of BC removed in tree biomass by harvesting will, in the long term, likely result in reduced soil BC pools and reduced BC leaching to streams. How much BC will be removed will depend on the type of trees (Olsson et al. 1996) and the intensity of harvesting. Mass-balance studies indicate that WTH will increase the depletion of base cations in forest soils in the long term (Joki-Heiskala et al. 2003; Duchesne and Houle 2006; Akselsson et al. 2007). However, soil mineral weathering rates are difficult to assess and there are large uncertainties (Ouimet and Duchesne 2005). Therefore, despite decades of concern about surface-water acidification, the empirical basis for defining forest harvest influences on acidity status is not a sufficient basis for reliable predictions about how a more intensified silvicultural practice, in combination with climate change, will affect the BC export from the soil to surface waters.

In a model study using MAGIC in Sweden, the long-term effect of forestry practice on ANC in stream runoff was quantified (Westling and Kronnäs 2006). The authors calculated the change in ANC in runoff after the next rotation period using different forestry scenarios. The study showed that the response to WTH would be small in Scots pine *(Pinus sylvestris)* stands, whereas the soils in Norway spruce (*Picea abies*) stands are more sensitive and could show a substantial decrease of ANC in runoff (Westling and Kronnäs 2006). In a typical spruce stand in northern Sweden, WTH would decrease ANC by 20 μequiv.·L^{-1} in runoff compared with a stem-harvesting scenario. The same calculations on a catchment in the southern part of Sweden show that a whole-tree utilization scenario would give a reduction of 45 μequiv.·L^{-1} ANC in runoff. In the most extreme site among the 12 MAGIC modeling sites, the difference in BC in runoff between whole-tree and stem-only harvesting was almost 100 μequiv.·L^{-1} (Westling and Kronnäs 2006). A major reason for the difference between the sensitivity of the northern and southern parts of the country is that the southern parts have been subject to a larger anthropogenic acidification (at present, ca. 5 kg S·ha^{-1}·$year^{-1}$ and 13 kg N·ha^{-1}·$year^{-1}$ deposition compared with ca. 1 kg S·ha^{-1}·$year^{-1}$ and 1.5 kg N·ha^{-1}·$year^{-1}$ in the northern study catchment; Björkvald et al. 2009). This has depleted exchangeable BC in the southern soils, leaving them more sensitive to further acidification caused by forestry.

There is no consensus on the degree to which intensified forestry will contribute to surface-water acidification. Most of the studies on the effect of WTH study BC saturation in

Fig. 3. The shaded bars indicate the observed drop in pH in the 60 stream segments from baseflow to spring snowmelt. Baseflow data from Buffam et al. (2008). The solid bars indicate the further decline in spring snowmelt pH associated with a reduction in BC of 30 μequiv.·L^{-1}.

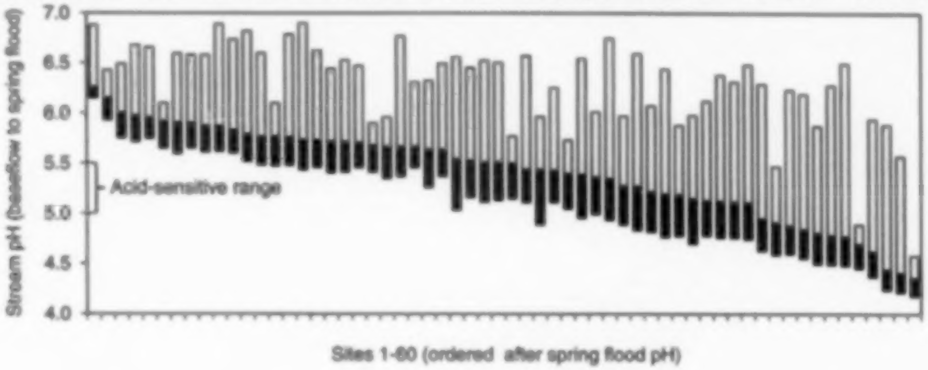
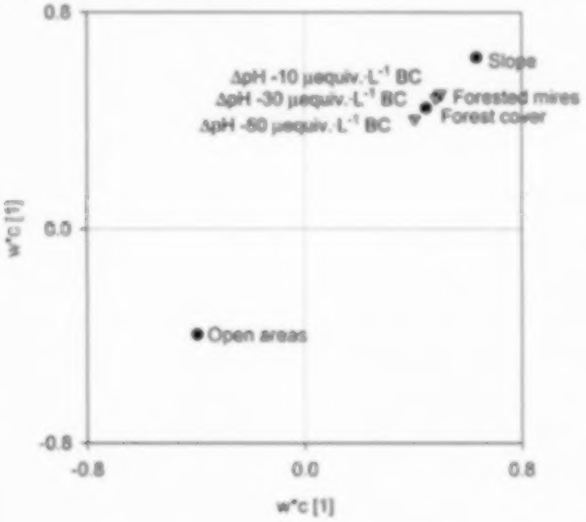


Fig. 4. Loading plot for the refined partial least squares (PLS) model with ΔpH from the different scenarios as dependent variables (shaded triangles) and four explanatory catchment characteristics variables (solid circles).



the soil, and few studies exist on runoff water. Comparison with long-term field experiments has shown that the soil hydrochemical MAGIC model might overestimate BC changes due to the lack of certain dynamic processes in the model. For instance, although second-rotation forest following WTH is expected to have a lower yield than the first rotation (in part due to soil BC depletion), this dynamic is not included in MAGIC. In long-term field experiments, the difference in acid load (in the soil) between stem-only and whole-tree utilization in measurements was only 10% of the potential (theoretical) effect (Staaf and Olsson 1991; Olsson et al. 2004). The evidence from experimental plots, mass-balance calculations, and modeling approaches indicates that intensified forestry will likely decrease BC in surface waters, but the quantification of that remains difficult. The response of stream BC dynamics to watershed BC depletion is expected to be complex and nonlinear, and we do not attempt to predict the dynamics of that response here. Rather, we present how sensitive stream pH is to stream BC change scenarios, of the same order of magnitude as those that might result from WTH. Based on literature studies, we would expect BC loss in streams to lie somewhere in the interval of 0 to 50 μequiv.·L^{-1}, but with variation between forest types (lower in pine and higher in spruce) and the location of the catchment (lower in the more pristine northern part of Sweden and higher in the more acidified areas in the south).

Impacts on stream pH distributions and ecological implications

During spring snowmelt, the pH drops in the study streams, and this study coupled with existing literature suggests that BC loss may result in further pH decline. Most of the pH drop (about 0.7–0.8 units) is due to natural variation resulting from dilution of ANC by the input of low ANC snowmelt water in combination with increasing levels of DOC (and associated organic acids) in the streams, which together typically result in the lowest annual pH (Bishop et al. 2000). At the end of the 1990s, about 0.1 to 0.2 pH units of the pH drop in the Krycklan catchment was due to acid deposition (Bishop et al. 2000); this part has now likely continued to decline as a result of the reduction in sulphur deposition (Laudon and Bishop 2002; Laudon and Hemond 2002). Spring snowmelt is the time when the streams would be most sensitive to BC removal because of the ANC decrease. In addition, during spring snowmelt, the water table rises, and the more superficial soil layers where we expect the largest WTH-derived changes in exchangeable BC (Olsson et al. 1996) contribute the majority of the snowmelt stream water (Bishop et al. 2004). In the scenarios, a removal of about 10–20 μequiv.·L^{-1} BC would affect pH of the same order of magnitude as acid deposition did at the end of the 1990s.

The study suggested that pH in the stream network was relatively sensitive to BC removal during spring snowmelt and that much of the stream length would decrease into the biologically sensitive range (pH 5–5.5) or below. Recent studies have shown that for many Swedish freshwaters, pH is the best predictor of brown trout mortality (Serrano et al. 2008), as well as the presence or absence of acid-sensitive fish species (Holmgren and Buffam 2005) and invertebrate

Fig. 5. Change in stream length with pH_{new} below or above the thresholds with different percentage removal of BC during the spring snowmelt.

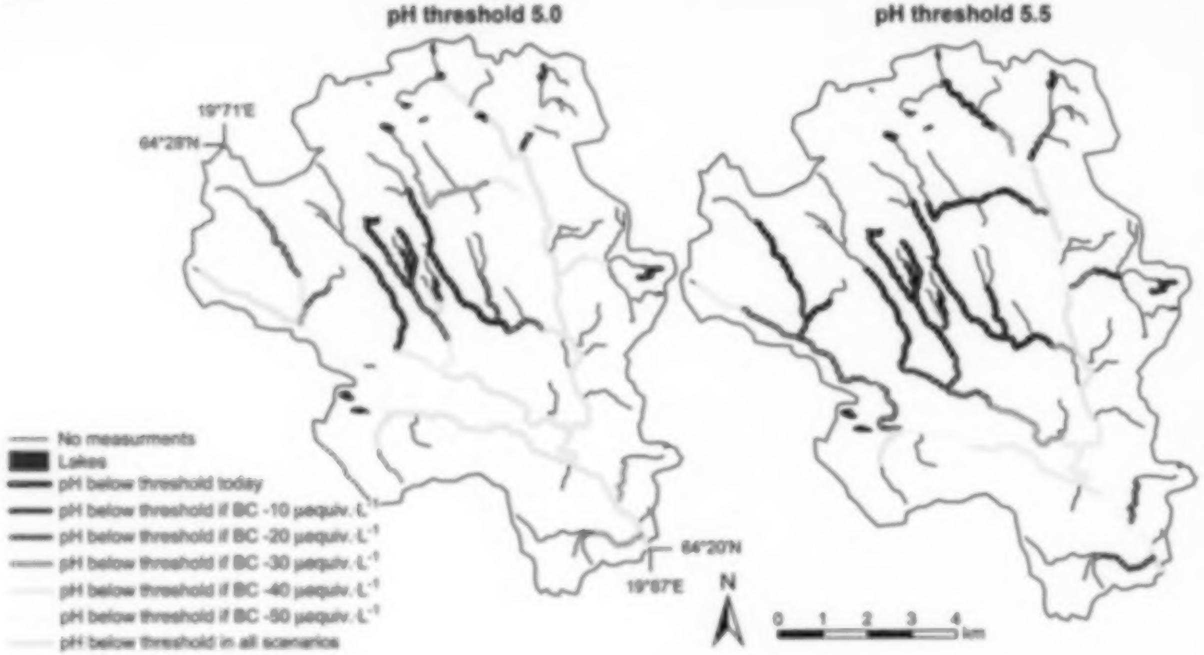
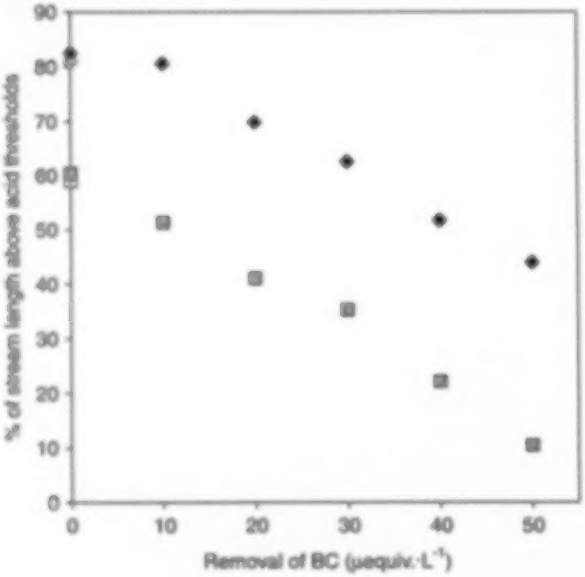

pH threshold 5.0
pH threshold 5.5
19°71'E
64°28'N
64°20'N
19°87'E
N
0 1 2 3 4 km
No measurments
Lakes
pH below threshold today
pH below threshold if BC -10 µequiv.·L⁻¹
pH below threshold if BC -20 µequiv.·L⁻¹
pH below threshold if BC -30 µequiv.·L⁻¹
pH below threshold if BC -40 µequiv.·L⁻¹
pH below threshold if BC -50 µequiv.·L⁻¹
pH below threshold in all scenarios

Fig. 6. Stream length affected by low pH during spring snowmelt in different BC removal scenarios: solid diamonds, stream length below pH threshold 5.0; shaded squares, stream length below pH threshold 5.5. To illustrate the model fit, we show both measured values (the open symbols) and modeled pH (solid symbols) on the y axis. Solid symbols indicate the stream length above acid thresholds calculated as pH_{new}.



species (Fölster et al. 2007). Although the spring snowmelt is a short period, it is extreme regarding pH changes and has been found to be an ecologically critical period for acid-sensitive biota (Lepori and Ormerod 2005). Removal of BC from the catchment is therefore likely to have an ecological impact on the habitats for a range of acid-sensitive aquatic species. According to the new Environmental Quality Guidelines from the Swedish Environmental Protection Agency, anthropogenic acidification at baseflow is classified as a human-induced pH drop of at least 0.4 units. However, during episodes, a change of 0.2 pH units is classified as anthropogenic acidification (Swedish Environmental Protection Agency (EPA) 2007). In the 30 µequiv.·L^{-1} removal scenario, that would mean that 58% of the stream length would be classified as anthropogenically acidified, and in the 50 µequiv.·L^{-1} scenario, 100% of the stream length would be classified as affected as a result of forestry effects alone.

pH was most easily affected between pH 5 and pH 6. Above pH 6, the bicarbonate becomes important for buffering pH changes, and for the lower pH range (below 5), the organic buffering becomes more significant. The sensitivity of pH to the BC-removal scenarios showed a clear spatial pattern in which downstream segments had a greater buffering capacity than the small headwaters. The PLS analysis supported the hypothesis that pH sensitivity was greatest in the small headwaters with a higher slope compared with the downstream areas with less topography. On the contrary, subcatchments with the open fields (agricultural areas on fine-grained soils) were better buffered and less sensitive to a removal of BC. This reflects the sensitivity of the catchments and the pH, BC, and ANC status of the different stream segments under present conditions (Buffam et al. 2008). The small, first-order catchments tended to be more acidic, whereas downstream catchments were better buffered. Stream pH has been found to vary with altitude and distance downstream in a predictable way in other studies as well (Likens and Buso 2006). This has been partly attributed to changes in flowpaths (Driscoll et al. 1988) where the downstream catchments, with a large accumulated stream length, will have a longer subsurface-water transit time. Hence, the groundwater will accumulate more ions from weathering of the mineral soils intersected en route to the stream. The downstream trend in pH can also be attributed to changes in soil types (Soulsby et al. 2006; Ågren et al. 2007; Buffam et al. 2008). The postglacial fine sediment (silty) area in the lower part of the catchment has a high specific surface area, which increases the opportunity for weathering and BC exchange between the mineral surfaces and the water. However, in the upper areas of the catchment, the soils are characterized by till, exposed bedrock, and thin soil (Ågren et al. 2007) and hence contain less BC that can be exported to the stream. The streams draining these upper areas are more prone to be adversely affected by BC removal. One notable exception is headwater wetland streams in which pH is largely buffered by high concentrations of organic acids. In our study, there was an overrepresentation (Buffam et al. 2008) of the downstream better-buffered catchments. Had we sampled more of the small, first-order catchments, it is possible that the increase in stream length with pH below biological thresholds would have been even greater in the different scenarios.

The PLS analysis also indicated that the subcatchments with a high proportion of forest cover and forested mires were more sensitive to a loss of BC. There is a large variation among catchments depending on soil types, distance downstream, and other variation, but there are plausible mechanistic reasons for the forest cover pattern. First, the lowest pH and BC are found in wetland areas and thus will not show a large pH change in our scenarios, partly due to the logarithmic scale of pH. At the other end of the scale, in streams with high pH and BC, the pH is buffered against change by bicarbonate (inorganic alkalinity). Many variables interact to create these high pH streams, but in brief, they are associated with agricultural areas, fine sorted sediments, lower lying stream reaches, and large accumulated stream length (Buffam et al. 2008). This means that downstream we get input of "old deep groundwater" into the streams that have had a long water subsurface transit time and also traversed the silty sediment area with a large specific area allowing for many ion exchange sites in the soil. Hence, this water is well buffered and rich in BC (Laudon et al. 2007; Buffam et al. 2008). Streams most sensitive to BC loss are instead those with upland forested catchments on unsorted till.

Although we have taken a simple approach with uniform BC removal in all streams for our scenarios, in reality, any response to an environmental perturbation would be more complex. The amount of BC that is removed from a catchment due to forestry would be affected by the forest coverage, tree species, different proposed rotation periods, stem-only or whole-tree harvesting, etc. The sensitivity of the catchment to the removal of BC is dependent on the weathering rates of the soils, which in turn are related to the Quaternary deposits. The hydrology of the catchment, the length of flowpaths, subsurface pathways, and residence time in the soil will also affect the sensitivity of the streams to catchment removal of BC, as will the historic acid deposition that may have reduced the base saturation of the soils (Jönsson et al. 2003). High nitrate fluxes have been observed in some studies following harvesting (Tremblay et al. 2009), and although this process only goes on for a couple of years after clear-cutting (Wang et al. 2006), the BC losses can be substantial during this period. Because we focus on the long-term perspective and nitrification is more short term, we decided not to go into this process any further. Also, the study region is severely nitrogen-limited, with very little leakage of nitrogen after clear-cutting (Löfgren et al. 2009). Future levels of acidifying pollutants may also affect the pH in the streams in the long term, even if the emissions have been reduced significantly. Nitrogen fertilization of forest soils to increase forest productivity is another scenario that might affect the pH in the streams, both due to increased removal of BC at harvesting and as a direct effect of nitrogen fertilization on nitrate fluxes. Reduced deposition, changed precipitation patterns, and higher temperatures may also affect the concentrations of organic acids in the streams in the future (De Wit and Wright 2008), as well as the weathering rate of BC (Egli et al. 2008). In the face of this complexity, we maintained a simple approach to testing the sensitivity of the stream network to BC loss, a likely effect of intensified forestry. The hope is that future research will provide a more detailed answer to the long-term effects that might be seen in stream pH, depending on the combination of factors listed above. Krycklan catchment is used as a case study, but the results here are probably informative for many sites with acid-sensitive soils in the northeastern US and Canada, as well as in Scandinavia where several modeling studies have predicted acidification with WTH (e.g., Joki-Heiskala et al. 2003; Clair et al. 2007; Aherne et al. 2008). Because the long-term effect of different forestry practices is unclear, there is all the more reason to evaluate BC sensitivity before, rather than after, eventual problems arise.

Acknowledgments

This project has been funded by FORMAS, SLU:s FOMA program on Acidification and the Soil and Water program of Future Forest.

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Long-term functional group recovery of lotic macroinvertebrates from logging disturbance

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Abstract: Clear-cut logging rapidly affects stream macroinvertebrates through substantial alteration of terrestrial–aquatic resource linkages; however, lesser known are the long-term influences of forest succession on benthic macroinvertebrate assemblages, which play key roles in stream ecosystem function. We compared secondary production and standing crops of detritus in two mountain headwater streams within Coweeta Hydrologic Laboratory, North Carolina, USA, as part of a long-term, paired-watershed investigation of macroinvertebrate recovery from whole-catchment logging. Mean annual habitat-weighted abundance and biomass in the disturbed stream (catchment entirely logged 26 years prior) did not differ from that of the reference stream, and secondary production was only 0.8 g ash-free dry mass (AFDM)·m^{-2}·$year^{-1}$ greater in the disturbed stream (disturbed, 10.1; reference, 9.3). Taxonomic composition, shredder–scraper ratios, and North Carolina biotic index scores were more similar between streams than in previous years. However, mean annual standing crops of benthic organic matter (BOM) were much lower in the disturbed stream (167 g AFDM·m^{-2}) than in the reference stream (339 g AFDM·m^{-2}), and the disturbed stream supported greater macroinvertebrate biomass per unit BOM. We suggest that despite similarities in macroinvertebrate structure and function, past logging activity continues to influence consumer–resource relationships in the disturbed stream through enhanced resource quality.

Résumé : La coupe forestière à blanc affecte rapidement les macroinvertébrés en modifiant de manière importante les liens entre les ressources terrestres et aquatiques; on connaît, cependant, moins bien les effets à long terme de la succession forestière sur les peuplements de macroinvertébrés benthiques qui jouent des rôles essentiels dans le fonctionnement des écosystèmes d'eau courante. Nous comparons la production secondaire et la biomasse du détritus dans deux cours d'eau d'amont de montagne au Coweeta Hydrologic Laboratory, Caroline du Nord, É.-U., dans le cadre d'une étude à long terme de bassins hydrographiques appariés sur la récupération des macroinvertébrés de la coupe de bois sur l'ensemble du bassin versant. L'abondance et la biomasse moyennes annuelles pondérées en fonction de l'habitat dans le cours d'eau perturbé (bassin entièrement soumis à la coupe 26 ans auparavant) ne diffèrent pas de celles du cours d'eau témoin et la production secondaire dans le cours d'eau perturbé est supérieure de seulement 0,8 g de masse sèche sans les cendres (« AFDM »)·m^{-2}·an^{-1} dans le cours d'eau perturbé (perturbé, 10,1; témoin, 9,3). La composition taxonomique, les rapports déchiqueteurs–racleurs et les cotes de l'indice biotique de Caroline du Nord sont plus semblables dans les cours d'eau que durant les années antérieures. Cependant, les biomasses moyennes annuelles de matière organique benthique (« BOM ») sont de beaucoup inférieures dans le cours d'eau perturbé (167 g AFDM·m^{-2}) que dans le cours d'eau témoin (339 g AFDM·m^{-2}) et le cours d'eau perturbé soutient une plus grande biomasse de macroinvertébrés par unité de BOM. Nous croyons que, malgré les similarités dans la structure et la fonction des macroinvertébrés, les activités de coupe forestière du passé continuent à influencer les relations consommateurs–ressources dans le cours d'eau perturbé par la qualité accrue des ressources.

[Traduit par la Rédaction]

Introduction

Stream ecosystem responses to the sudden removal of watershed vegetation (i.e., clear-cut logging) are well studied, and because of the widespread use of benthic fauna as bioindicators of anthropogenic disturbance, much attention is paid to influences on macroinvertebrate assemblages (e.g., Fuchs et al. 2003). Logging activities that do not leave riparian vegetation (i.e., buffers) intact can induce a short-term shift in the energy base of streams from allochthonous sources of carbon to autochthonous in-stream production (Webster et al. 1983; Wallace and Gurtz 1986), which then declines with canopy regrowth during forest succession. These short-term changes are often reflected in macroinvertebrate assemblage structure as shifts in relative abundance and biomass among functional feeding groups (FFGs) (Wal-

Received 1 September 2009. Accepted 27 March 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 18 June 2010. J21377

Paper handled by Associate Editor John Richardson.

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Can. J. Fish. Aquat. Sci. 67: 1126–1134 (2010) doi:10.1139/F10-045 Published by NRC Research Press

lace et al. 1988). However, little is known about the long-term (i.e., >10 years) influences of watershed logging on benthic macroinvertebrate assemblages, which play key roles as organic matter processors and intermediaries in aquatic food webs (Wallace and Webster 1996).

Long-term models of stream resource dynamics during forest succession predict reduced inputs of dam-forming wood, resulting in greater material transport and reduced retention of benthic organic matter (BOM) and inorganic nutrients until late successional stages (Webster et al. 1992; Valett et al. 2002). In addition, the quality of allochthonous detritus may change with the composition of the aggrading forest (Boring et al. 1981). Thus, in contrast to brief, pulse-type disturbances that can alter habitat structure and cause mass mortality, watershed logging represents a press-type disturbance (sensu Bender et al. 1984) to stream ecosystems with long-term consequences for the quantity, quality, and storage of resources.

Benthic macroinvertebrate assemblage structure and function are contingent upon the nature and relative availability of resources, and strong positive associations exist between detrital resource standing crops and secondary production in forested headwater streams (Wallace et al. 1999). The tight relationships between macroinvertebrate consumers and resource type and delivery are represented by the morphological and behavioral adaptations for food acquisition that describe macroinvertebrate FFGs (i.e., scrapers, shredders, collector–gatherers, collector–filterers, and predators; Cummins 1973). Shifts in the relative proportions of FFGs reflect changes in resource dynamics, and specific FFG ratios have been proposed as surrogates for various ecosystem attributes (Merritt et al. 2002). Thus, long-lasting, resource-related influences of forest succession on stream ecosystem condition could be evident in the relative amounts of biomass and production found among FFGs. However, extended temporal changes in forested headwater assemblages following logging are poorly understood, as most studies (with the exception of Stone and Wallace (1998), discussed below) either focus on short-term responses or compare multiple streams draining stands of different ages. Furthermore, long-term patterns in functional attributes of benthic macroinvertebrates within a particular stream ecosystem following logging are relatively unknown.

Recently, investigations of ecosystem function have been recommended to accompany traditional, structural-based metrics (i.e., species distribution and abundance) in the assessment of stream response to catchment disturbance (Young et al. 2008). Collective properties of ecosystems integrate environmental conditions with biological patterns, thus providing relative information on the status of a broad range of system attributes (Young et al. 2008). The elaboration of consumer biomass (i.e., secondary production) is an energetic function of stream ecosystems that reflects thermal regime, resource availability, and water quality and is a measure of the relative success of assemblages that takes into account numerous taxon-specific differences in life history (Huryn and Wallace 2000; Benke and Huryn 2006). Studies of secondary production have enhanced our understanding of the energetic relationships between streams and other watershed subsystems (e.g., lake outlets; Wotton 1988) and have illustrated ecosystem-level sensitivity to changes in resource (Wallace et al. 1999) and nutrient (Cross et al. 2006) loading, as well as broad differences in land use (Shieh et al. 2003). Because macroinvertebrate assemblages in forested headwater streams rely mainly on allochthonous detritus to meet their energetic demands, investigations of secondary production coupled with a knowledge of benthic resource dynamics (i.e., inputs, standing stocks) are particularly effective tools in evaluating stream response and recovery following catchment-scale disturbances or anthropogenic alterations such as logging.

In the present study, we investigated benthic resource pools and annual whole-assemblage secondary production in a stream draining a previously logged (26 years prior) catchment and its paired reference as part of a long-term assessment of benthic macroinvertebrate recovery from logging disturbance; this paired-watershed study remains the longest-running investigation of stream ecosystem response to clear-cut logging in North America. We compared various structural attributes of macroinvertebrate assemblages (abundance, biomass, taxonomic composition, and biotic indices), mean annual secondary production of macroinvertebrate FFGs, and standing crops of detritus between the previously disturbed stream and its long-term reference to evaluate the current recovery status of the logged stream. We also compared our findings with those of previous investigations of these systems to gain perspective on the relative magnitudes and trends in resource availability and macroinvertebrate response.

Materials and methods

Study sites

The study sites are two perennial second-order streams within the Coweeta Hydrologic Laboratory (US Forest Service) in Macon County, North Carolina, USA. Coweeta is a 1625 ha drainage basin located in the Blue Ridge Physiographic Province of the southern Appalachian Mountains. The Coweeta basin receives 187 cm of precipitation annually and is drained by high-gradient headwater streams composed of bedrock-outcrop, riffle, and depositional habitats. Coweeta streams are highly heterotrophic: primary production is extremely low (~6 $g \cdot m^{-2} \cdot year^{-1}$), and terrestrial detrital inputs are ~400 $g \cdot m^{-2} \cdot year^{-1}$ (Webster et al. 1997).

The reference (Hugh White Creek) and disturbed (Big Hurricane Branch) streams were paired for study because of their many physical similarities (Table 1), and although quantitative pretreatment data are lacking, the macroinvertebrate communities of both streams were observed to be similar before logging (Gurtz and Wallace 1984). Prior to logging, both catchments were dominated by mixed hardwoods, including oaks (*Quercus* spp.), hickory (*Carya* spp.), red maple (*Acer rubrum* L.), and yellow poplar (*Liriodendron tulipifera* L.). After the 1977 cable logging of the entire catchment (except 16 ha on upper slopes and ridges), forest succession was allowed to progress undisturbed. Leaf area index (a measure of canopy cover) of the logged catchment (Elliott et al. 2002) and litter inputs to the disturbed stream (Webster et al. 1992) recovered within 10 years of deforestation. Recent forest inventories on the logged site report more basal area of black locust (*Robinia pseudoacacia* L.), *L. tulipifera*, and *A. rubrum* and a distinct lack of

Table 1. Physical characteristics of reference (Hugh White Creek) and disturbed (Big Hurricane Branch) streams located within the Coweeta Hydrologic Laboratory, North Carolina, USA.

	Reference	Disturbed
Catchment orientation	Northwest	South
Catchment area (ha)	61	59
Reach length (m)	1077	1225
Maximum channel elevation (m asl)	992	1077
Elevation at weir (m asl)	707	722
Mean annual temperature (°C)[*]	10.8	11.2
Mean discharge $(L \cdot s^{-1})$[†]	29.9	27.7
Habitat composition		
Bare bedrock (%)	12.1	9.6
Mossy bedrock (%)	14.5	11.5
Riffle (%)	38.9	48.2
Depositional (%)	34.6	30.7

Note: asl, above sea level.

[*] 10 February 2003 to 9 February 2004.

[†] 1 January 2003 to 1 September 2003.

oak and hickory species (Elliott et al. 2002). A dense understory of rhododendron (*Rhododendron maximum* L.) provides year-round shading of both stream channels. Further details of the 1977 clear-cut were reported by Swank et al. (2001) and Elliott et al. (2002).

Previous investigations of macroinvertebrate response to logging in this system have revealed two major patterns: a sudden increase in scraper production associated with a temporary period of high algal growth (Wallace and Gurtz 1986), and a delayed, long-term increase in shredder production following the return of leaf inputs (Stone and Wallace 1998). Sixteen years after logging, Stone and Wallace (1998) were the first to compare whole-assemblage secondary production rates between these streams. Macroinvertebrate abundance, biomass, and production were all greater in the disturbed stream at that time, yet no compositional differences were found between assemblages using multiple biotic indices (Stone and Wallace 1998).

We are aware of the statistical limitations associated with this unreplicated design (Hurlbert 1984). Nonetheless, as with previous investigations of this system, we adhere to the tradition of the paired-watershed approach (Bormann and Likens 1979), which has advanced our understanding of large, complex systems (Schindler 1998).

Benthic sampling

Benthic sampling for the current study began in February 2003 and continued every other month through December 2003. Four habitats (bare bedrock, moss-covered bedrock, riffle, and depositional areas), each within upper, middle, and lower sections of both streams (i.e., $n = 3$ per habitat; Table 1), were sampled randomly on each date. A stovepipe corer (area = 400 cm^2) was used to sample depositional habitats. All benthos within the corer was removed up to a depth of 10 cm and poured through a 250 µm mesh bag. Riffle samples were collected using a Surber sampler (0.093 m^2) with a 250 µm mesh net. Bare and moss-covered bedrock habitats were scraped (100 cm^2) with a dull knife and then scrubbed with a nylon brush into a 250 µm mesh bag. All samples were preserved in the field using a 37% formalin solution containing Phloxine B dye to facilitate invertebrate sorting in the laboratory.

Current velocity (Gessner bag current meter; Gessner 1950) and substrate size class proportions (visual inspection) were measured prior to collecting each sample. We used the Wentworth scale of substratum size classification, and sizes were converted to a phi scale (negative log base 2); the median φ for each sample was then calculated (Cummins 1962). The US Forest Service provided mean daily discharge and temperature for both streams (available online: http://coweeta.uga.edu/ecology/ecology_data.html).

Sample processing and secondary production

In the laboratory, samples were poured through 1 mm and 250 µm nested sieves creating coarse and fine fractions, respectively. Invertebrates were removed from benthic material by hand under a dissecting microscope at 15× magnification. All invertebrates were then identified and counted, and their lengths were measured to the nearest millimetre. Most identification was to genus using the keys of Merritt and Cummins (1996) and Wiggins (1977). Midge larvae (Diptera: Chironomidae) were identified as either Tanypodinae (predaceous) or non-Tanypodinae (non-predaceous); non-insect taxa were identified to order. Each taxon was assigned a FFG according to Merritt and Cummins (1996), Stone and Wallace (1998), and Wallace et al. (1999).

Macroinvertebrate biomass (ash-free dry mass, AFDM) was calculated using length–weight regressions from Benke et al. (1999). Cohort production intervals for each taxon were obtained from previous studies (Stone and Wallace 1998; Wallace et al. 1999). Secondary production ($g\ AFDM \cdot m^{-2} \cdot year^{-1}$) for most taxa was calculated using the size-frequency method as outlined by Benke and Huryn (2006). An empirically derived production-to-biomass ratio ($P{:}B$) of 18 was used for Copepoda (see Wallace et al. 1999), and a $P{:}B$ of 5 was used for Nematoda, Oligochaeta, and Turbellaria (Benke et al. 1984). Rare taxa were assigned $P{:}B$ values of 5 and 10 for univoltine and bivoltine taxa, respectively (Lugthart and Wallace 1992); production for these taxa was calculated by multiplying $P{:}B$ by mean annual biomass. *Micrasema* (Trichoptera: Brachycentridae) abundance, biomass, and production were divided evenly between the gatherer and shredder functional groups (Wallace et al. 1999). Secondary production of each taxon (with the exception of crayfish (*Cambarus* spp.) and salamanders (*Desmognathus* and *Eurycea* spp.), which were not considered because of inadequate sampling procedures and the increased nocturnal activity of some organisms) was estimated within each habitat. We calculated habitat-weighted values of macroinvertebrate abundance, biomass, and production by summing habitat-specific areal values multiplied by the proportion of total reach length occupied by each habitat type (Table 1). To estimate habitat proportions, we visually inspected each consecutive 5 m segment of both stream reaches.

Benthic organic matter

To determine BOM composition and biomass, samples were poured (following invertebrate removal) through 4.75 mm, 1 mm, and 250 µm sieves. Organic matter ≥

4.75 mm was sorted to leaves, wood, and miscellaneous coarse benthic organic matter (CBOM). All organic matter ≥1 mm was designated miscellaneous CBOM, and particles smaller than 1 mm but larger than 250 μm were considered fine benthic organic matter (FBOM). The AFDM of all OM types was calculated as the difference between dried (50 °C, 7 days) and ashed (500 °C, 24 h) mass.

Among-year invertebrate comparisons

Differences in sample collection methods and variables measured among the study years prevented the direct comparison of macroinvertebrate and BOM data among studies. The 1977 (Gurtz and Wallace 1984) and 1982 (Wallace et al. 1988) studies used a Surber sampler in both riffle and depositional habitats and quantified only invertebrate abundance. Furthermore, the 1982 study considered taxa only from the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). In 1993, a different sampling method was used for each habitat, and the use of a stovepipe corer in depositional areas enhanced the recovery of small, early-instar taxa compared with previous studies (Stone and Wallace 1998).

To assess taxonomic similarity between EPT assemblages, we calculated a single Bray–Curtis index score using mean annual taxon-specific abundance for each study year as

$$\mathrm{BC} = \frac{\sum_{i=1}^{S} |y_{id} - y_{ir}|}{\sum_{i=1}^{S} (y_{id} + y_{ir})}$$

where BC is the Bray–Curtis distance between the reference and disturbed streams, y_{id} is the abundance of taxon i at the disturbed stream d, y_{ir} is the abundance of taxon i at the reference stream r, and S is the total number of EPT taxa present at the disturbed and reference sites (Krebs 1999). The BC index coefficient attains a maximum value of 1 for two sites that are entirely different and a minimum value of 0 for two sites with identical assemblages.

We used two biotic indices found by Stone and Wallace (1998) to have the greatest ability to detect differences between assemblages in logged and unlogged streams. The shredder–scraper ratio (shredder abundance / scraper abundance) and the North Carolina biotic index (NCBI; Lenat 1993; also described in detail in Stone and Wallace 1998) were calculated using EPT abundance for qualitative comparison with the previous three studies.

Statistical analyses

To account for seasonally driven patterns, paired t tests ($\alpha = 0.05$) on habitat-weighted means from upper, middle, and lower reaches on each sampling date ($n = 6$) were used to detect significant differences between streams for mean annual BOM standing crop, mean annual macroinvertebrate abundance and biomass, shredder–scraper ratios, and NCBI scores. Mean habitat-specific current velocities were compared using a paired t test ($n = 6$), and differences in mean habitat-specific substrate median φ values were evaluated with a t test ($n = 6$). To examine seasonal patterns of primary resource standing crops (leaves + CBOM + FBOM) between streams, we conducted t tests on each sampling date using mean habitat-weighted values from upper, middle, and lower reaches ($n = 3$ per stream). To examine the relationship between consumers and benthic resource availability, we used linear regression of invertebrate biomass with primary BOM resource standing crop from individual Surber and core samples in riffle and depositional habitats, respectively, collected on all dates ($n = 36$ per stream).

To address changes in resource availability in both streams over the decade between 1993 and the current study, habitat-weighted mean annual standing crops of primary BOM resources were calculated for both streams in both studies and were compared using two-way analysis of variance (ANOVA) on mean values from each sampling date ($n = 6$ for both streams in both study years) with year and stream as main effects. Tukey's honestly significant difference (HSD) was used as a post-hoc multiple comparison test following significant ANOVA results. Data failing normality tests were $\log(x + 1)$-transformed. All statistical analyses were performed using SigmaStat (version 3.11, Systat Software Inc., Chicago, Illinois).

Results

Physical measurements

Because of weir repair in autumn, comparisons of stream discharge during 2003 were limited to January through early September. Mean discharge was slightly higher in the reference stream (Table 1); hydrographs from both streams displayed nearly identical patterns (data not shown). Mean annual temperatures were also similar between streams (reference, 10.8 °C; disturbed, 11.2 °C; Table 1); however, the annual temperature range in the disturbed stream (12.1 °C) was less than in the reference stream (16.2 °C) and resulted in 164 more (or 4.2% greater) degree-days above 0 °C accumulated in the disturbed stream. Habitat-specific current velocities and substrate sizes did not differ between streams for any habitat (paired t test, $P > 0.05$, $n = 6$).

Invertebrate assemblages

Annual habitat-weighted means of total macroinvertebrate abundance and biomass did not differ between streams (Table 2). Likewise, we found no differences in abundance or biomass comparisons within any FFG between streams (Table 2).

Habitat-weighted secondary production within both streams was estimated for a total of 70 taxa. Annual macroinvertebrate production was similar between the disturbed (10.1 g $\mathrm{AFDM \cdot m^{-2} \cdot year^{-1}}$) and reference (9.3 g $\mathrm{AFDM \cdot m^{-2} \cdot year^{-1}}$) streams, with nearly identical contributions by all FFGs (Table 2). In both streams, production was greatest for shredders and least for scrapers (Table 2).

Biotic indices indicated similar assemblage composition between streams. Using abundance of EPT taxa, no differences between NCBI scores (reference, 1.59; disturbed, 1.72; paired t test, $P = 0.19$, $n = 6$) or shredder–scraper ratios (reference, 3.97; disturbed, 4.82; paired t test, $P = 0.78$, $n = 6$) were found between streams. The reference assemblage included three taxa (*Anchytarsus*, *Habrophlebia*, and *Psilotreta*) not found in the disturbed stream, whereas the disturbed assemblage had only one unique taxon (*Taeniopteryx*). Taxonomic dissimilarity calculated using

Table 2. Mean annual habitat-weighted abundance (number·m^{-2} ± standard error, SE), biomass (mg ash-free dry mass·m^{-2} ± SE), and secondary production (mg ash-free dry mass·m^{-2}·year^{-1}) of macroinvertebrate functional feeding groups (FFG) in reference and disturbed streams.

	Abundance		Biomass		Production	
FFG	Reference	Disturbed	Reference	Disturbed	Reference	Disturbed
Scrapers	653±218	922±95	71±17	78±23	457	498
Shredders	1 764±460	1 701±358	561±196	582±144	2717	2 786
Gatherers	44 556±4530	41 104±3701	295±57	325±62	2440	2 530
Filterers	913±422	1 245±525	281±82	324±64	2050	2 310
Predators	3 574±321	4 020±591	432±105	618±106	1612	1 988
Total	51 460±5093	48 990±3998	1639±326	1927±212	9276	10 111

Note: No significant differences in abundance or biomass were found between streams within any FFG category or for total amounts (paired *t* test, $\alpha = 0.05$, $n = 6$).

the BC distance was 0.55 in 1977, 0.48 in 1982, 0.28 in 1993, and 0.19 in 2003 for an overall decline of 65% over the 26-year period.

Benthic organic matter

Mean annual habitat-weighted standing crops of BOM were two times higher in the reference stream (339 g AFDM·m^{-2}) than in the disturbed stream (167 g AFDM·m^{-2}; paired *t* test, $P = 0.028$, $n = 6$) (Table 3). Greater amounts of all BOM types were found in the reference stream, but the relative contribution of each BOM type to total standing crop was similar between streams (Table 3). Primary resource standing crops in the disturbed stream were significantly lower than in the reference stream ($P < 0.05$, *t* test, $n = 3$) on all dates except August and displayed diminished seasonal peaks during spring and autumn (Fig. 1).

Consumer–resource relationships

Benthic macroinvertebrate biomass was positively related to the quantity of primary BOM resources in both streams (reference, $r^2 = 0.59$, $P < 0.001$; disturbed, $r^2 = 0.62$, $P < 0.001$; $n = 36$) (Fig. 2). The slope of this relationship was greater in the disturbed stream (0.405) than in the reference stream (0.272; *t* test of slopes, $P = 0.035$; Zar 1999).

Both benthic resources and secondary production doubled in the reference stream from 1993 to 2003, whereas in the disturbed stream, OM standing crop and secondary production slightly decreased and increased over the decade, respectively (Table 4). The combined mean annual standing crop of leaves, CBOM, and FBOM was similar between streams in 1993, and OM standing crop in the disturbed stream during 2003 was not different from that found in 1993 (Tukey's HSD, $P > 0.05$; Table 4). BOM resources in the reference stream during 2003 were greater than in 1993 (Tukey's HSD, $P = 0.004$) and were also greater than those found in the disturbed stream in either year (two-way ANOVA, year × site interaction, $P = 0.004$; Table 4).

Discussion

Macroinvertebrate assemblage comparisons

The structure and function of the macroinvertebrate assemblages in these two streams were more similar in this study than in any previous investigation. Though we cannot compare annual production statistically, total annual habitat-weighted production in the current study was only 9% greater in the disturbed stream compared with the 190% greater annual production (vs. the reference) found 10 years prior (Stone and Wallace 1998). Production of each FFG in 2003 differed by only 3%–13% between streams, except for predators, which had 23% greater production in the disturbed stream. Although greater predator production was mostly due to differences on bedrock outcrops, predators contributed similarly to total production in both the reference (17%) and the disturbed (20%) streams. Because secondary production accounts for taxon-specific characteristics such as life span, survivorship, and fecundity (Huryn

Table 3. Mean annual habitat-weighted benthic organic matter (BOM) standing crops (g ash-free dry mass·m^{-2} ± standard error, SE) in reference and disturbed streams.

BOM type	Reference	Disturbed	*P* value*
Wood	135±25	74±15	0.039
Leaves	45±15	22±7	0.075
CBOM	86±20	32±7	0.010
FBOM	72±11	39±10	0.023
Total	339±69	167±27	0.028

Note: CBOM, coarse BOM; FBOM, fine BOM.

*Paired *t* test, $n = 6$.

Fig. 1. Mean habitat-weighted benthic organic matter (leaves and coarse and fine fractions of benthic organic matter only) on each sample date in reference (solid circles) and logging-disturbed (open circles) streams. Points are means ± standard error (SE). *, $P \le 0.05$; **, $P \le 0.01$; *t* test, $n = 3$. AFDM, ash-free dry mass.

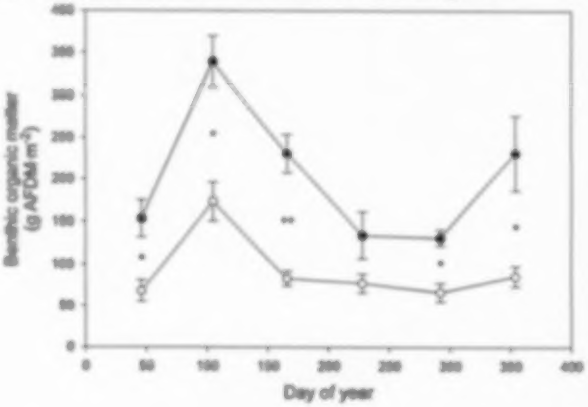

Fig. 2. Linear regressions of macroinvertebrate biomass and benthic organic matter resources (wood not included; riffle and depositional habitats only) in reference (solid circles, continuous line; $r^2 = 0.59$, $P < 0.001$, $n = 36$) and disturbed (open circles, broken line; $r^2 = 0.62$, $P < 0.001$, $n = 36$) streams. Slopes are significantly different (t test, $P = 0.035$). AFDM, ash-free dry mass.

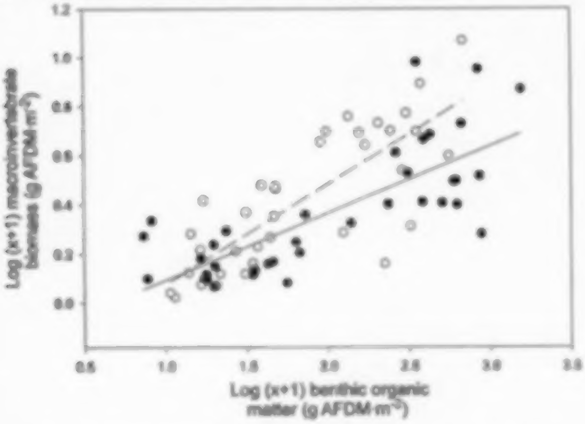

Table 4. Mean annual habitat-weighted benthic organic matter (BOM) standing crops (leaves, coarse BOM, and fine BOM only) and secondary production in reference and disturbed streams in 1993 and 2003.

	BOM ($g\ AFDM{\cdot}m^{-2}$)		Secondary production ($g\ AFDM{\cdot}m^{-2}{\cdot}year^{-1}$)	
	1993	2003	1993	2003
Reference	99.5a	203.5b	4.4	9.3
Disturbed	137.3a	92.6a	8.3	10.1

Note: Values in the BOM category that share a letter are not significantly different (two-way analysis of variance (ANOVA); Tukey's honestly significant difference (HSD); $\alpha = 0.05$; $n = 6$). BOM data for 1993 are from M.K. Stone (Georgia Perimeter College, Science Department, Dunwoody, Georgia 30338, USA, unpublished data). AFDM, ash-free dry mass.

and Wallace 2000), similar secondary production between streams suggests that the activity and success of the disturbed assemblage has converged with the reference assemblage over the 26-year period.

Biotic indices also imply convergence in species composition. Bray–Curtis dissimilarity, which considers both the richness and evenness of the assemblage, consistently declined with each investigation. Likewise, NCBI scores became increasingly similar since logging. The NCBI incorporates tolerance values (i.e., higher scores indicate a greater proportion of taxa tolerant to disturbed conditions; Lenat 1993) into a measure of diversity. The steady decline of this score in the disturbed stream since logging (Stone and Wallace 1998; present study) suggests that logging influences have consistently lessened over time, allowing more sensitive taxa to persist. Decreases in NCBI scores are also associated with the recovery of leaf decomposition, organic matter dynamics, and secondary production (Wallace et al. 1996); thus the consistent decline in NCBI scores is indicative of the ongoing functional recovery of this system.

Changes in the shredder–scraper ratio since logging reflect shifts in stream energy base and indicate a nonlinear recovery trajectory of the dominant detritivores. During 26 years, the EPT-based shredder–scraper ratio was consistently between 2.7 and 4.0 in the reference stream. In the disturbed stream, the ratio was 0.33 and 0.81 in 1977 and 1982, respectively, due to the immediate rise and subsequent decline in algae-scraping mayflies (Wallace and Gurtz 1986; Wallace et al. 1988). Sixteen years following logging, scraper abundance was similar but shredder densities were much higher in the disturbed stream, where the habitat-weighted shredder–scraper ratio was 8.0 (Stone and Wallace 1998). In the current study, no differences in abundance or biomass were found for shredders or scrapers and shredder–scraper ratios were more similar than any previous year, differing only 0.8 between streams. However, although these assemblage-based assessments suggest recovery of various ecosystem dynamics (e.g., primary production, decomposition, and species interactions), the increased similarities in these assemblages compared with a decade before were due to changes in the reference assemblage. We contend that OM dynamics explain these changes and indicate continued differences in consumer–resource relationships not apparent from macroinvertebrate data alone.

Benthic organic matter storage

From 1993 to 2003, BOM doubled in the reference stream but did not change appreciably in the disturbed stream. In the 4-year period preceding both studies, mean annual precipitation was 17% greater than the long-term (72 years) mean at Coweeta before 1993 and 16% lower before 2003 (Coweeta Long Term Ecological Research: http://coweeta.ecology.uga.edu). Wet and dry periods often drive BOM depletion and accumulation, respectively (Wallace et al. 1995; 1997), and may explain the increase in BOM that we observed in the reference stream. Further, the lack of change in BOM standing crops in the disturbed stream despite differences in precipitation patterns suggests an impairment of retentive ability.

We suspect a decreased ability to retain particulate materials in the disturbed stream from our observations of lower OM standing crops throughout the year, despite equivalent annual inputs (J.R. Webster, Virginia Tech, Department of Biology, Blacksburg, Virginia, unpublished data). BOM standing crops were consistently lower in the disturbed stream on almost all sampling dates and did not display the peak in late autumn found in the reference stream during this study, a pattern also observed by Golladay et al. (1989). Greater macroinvertebrate ingestion is most likely not the cause of lower BOM in the disturbed stream because (*i*) this would lead to higher rates of production, and (*ii*) increases in ingestion would be unreasonably high to mask the autumnal pulse of OM inputs. We also observed similar contributions of each OM type to total standing crops in both streams (i.e., indiscriminate export), suggesting reduced physical retention. Fewer debris dams have been reported for the disturbed stream previously (Golladay et al. 1989), but current inventories of debris dams and measurements of particulate OM retention are needed.

Lower BOM standing crops in the disturbed stream may also be due to enhanced microbial decomposition under elevated nutrient concentrations. Nitrate-N concentrations have been 3–10 times higher in the disturbed stream than in the reference stream since deforestation (Swank et al. 2001), and mean annual nitrate-N concentration in the disturbed stream during the current study year (114 $\mu g \cdot L^{-1}$) was almost 12 times that of the reference stream (10 $\mu g \cdot L^{-1}$; J.D. Knoepp, USDA Forest Service, Coweeta Hydrologic Laboratory, Otto, North Carolina, unpublished data). Nutrient enrichment positively affects microbial biomass (Meyer and Johnson 1983) and breakdown rates of leaf litter (Greenwood et al. 2007), and microbial growth may be further enhanced by the high N and P content of early-successional leaf litter (Boring et al. 1981). Enhanced microbial activity was suggested by Benfield et al. (2001) as partly responsible for faster leaf breakdown rates found in the disturbed stream. Thus, the lower amounts of BOM that we observed are probably due to a combination of reduced physical retention, a greater proportion of early-successional leaf litter, and increased microbial activity due to nutrient enrichment.

Consumer–resource relationships

Because of the energetic dependence of headwater macroinvertebrate assemblages on allochthonous inputs, secondary production increases with detritus standing crops in temperate, forested streams (Wallace et al. 1999). Comparing the 1993 and 2003 studies, this relationship appears to be true within each of our study streams: secondary production in the reference stream increased twofold along with a doubling of BOM, whereas in the disturbed stream, neither BOM nor secondary production changed appreciably. Furthermore, we found strong positive associations between macroinvertebrate biomass and BOM standing crop in both streams. However, in both of these analyses, there appears to be greater resource-use efficiency in the disturbed stream (see also Greenwood et al. 2007). In 1993, secondary production in the disturbed stream was double that of the reference stream despite similar BOM quantities, and currently, abundance, biomass, and production in the disturbed stream are similar to those in the reference stream despite half the amount of BOM. Additionally, there is currently greater macroinvertebrate biomass per unit BOM standing crop in the disturbed stream.

Resource quality may be germane to consumer–resource relationships in both streams, as the quantities of litter entering the streams appear to be in excess of macroinvertebrate requirements. Using rough estimates of macroinvertebrate assimilation efficiency (AE) of leaf detritus and net production efficiency (from Benke and Wallace 1997), the amount of ingestion required to support observed shredder, gatherer, and filter-feeder production was between 19%–21% of OM inputs in either stream (D.T. Ely, unpublished data). Although this is an extremely coarse evaluation, these values are similar to calculations of ingestion in headwater streams elsewhere (Rosi-Marshall and Wallace 2002). Although export, respiration, and burial may all be significant pathways of resource loss, calculated annual ingestion was only about 33% and 77% of the mean annual standing crop of BOM resources in the reference and disturbed streams, respectively. These findings suggest that a specific subset of high-quality BOM is driving the observed relationships between BOM and macroinvertebrate biomass and growth.

The notion of a higher-quality subset of the OM pool is consistent with concepts of proper conditioning and the palatability of resources, which are microbially mediated phenomena. Because neither individual body sizes (biomass/abundance) nor assemblage growth rates (production/biomass) differ between streams, we hypothesize that macroinvertebrates in the disturbed stream display comparable growth despite lower resource abundance because these resource pools contain a larger subset of high quality OM that is more easily assimilated by detritivores. If higher nutrient concentrations have increased microbial biomass, as found in similar streams (Meyer and Johnson 1983), then greater macroinvertebrate AE of microbe-rich OM is logical. Invertebrate AE is much higher for microorganisms than for detritus (Cummins and Klug 1979), and greater microbial abundance in the disturbed stream should increase the size of the palatable resource pool in addition to providing some of the carbon responsible for comparable production to the reference stream. Hutchens et al. (1997) measured the AE of *Pycnopsyche*, a predominant leaf-shredding caddisfly, on leaves from both streams and found that AE depended only on leaf conditioning time, regardless of stream identity. However, both the laboratory and field experiments in that study used leaves conditioned in the reference stream only, which prevented any effect due to differences in microbial colonization. Determining the AE of consumers on substrates conditioned in their respective streams should be a future priority and may show that nutrient enrichment can counteract reductions in BOM retention imposed by forest succession by lowering macroinvertebrate dependence on resource quantity, as found in an experimental nutrient enrichment study in a nearby stream (Cross et al. 2006; Greenwood et al. 2007).

We caution against the sole use of macroinvertebrate metrics in the assessment of lotic recovery from large-scale disturbances because such events can influence numerous structural and functional attributes of terrestrial and aquatic systems for decades. Although we have not directly shown that consumer–resource relationships remain impaired in the third decade since logging, the disturbed stream displays many characteristics that suggest enhanced resource quality. We measured only one of many stream ecosystem processes that may be influenced by forest succession. A more thorough understanding of both the recovery of benthic macroinvertebrates from logging disturbance and their role in the recovery of the system as a whole may be achieved via the simultaneous measurement of energy- and nutrient-related functions such as ecosystem metabolism, nutrient uptake, organismal growth rates, and litter decomposition, in addition to secondary production.

Finally, macroinvertebrate dependence on allochthonous resources may limit the application of general successional theory to stream assemblages, a topic that receives continued attention (Milner et al. 2008). Whereas forest succession is an autogenic process characterized by interacting, self-organized mechanisms of facilitation, inhibition, and tolerance (Connell and Slatyer 1977), lotic consumer recovery in donor-controlled systems (sensu Polis et al. 1997) is largely an allogenic function more dependent on interactions across system boundaries than within the assemblage. To better understand the recovery process of logged stream ecosystems, future studies should focus on the role of forest succession in determining inputs, retention, resource quality and quantity, and how these variables interact to influence the structure and function of macroinvertebrate assemblages.

Acknowledgements

We thank Amanda Ely, Wyatt Cross, and Sue Eggert for help in both the field and laboratory. This research was supported by the National Science Foundation under Cooperative Agreements DEB-9632854, DEB-0212315, and DEB-0218001 and the Coweeta LTER Program. Any opinions, findings, conclusions, or recommendations expressed in the material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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A multispecies age-structured assessment model for the Gulf of Alaska

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Abstract: Predation is the largest source of mortality for marine fish and may be an important process in regulating population size. Recent population models have attempted to quantify predation separately from other sources of natural mortality. Building upon such work, a multispecies age-structured assessment model (MSASA) for the Gulf of Alaska was developed, which included arrowtooth flounder (*Atheresthes stomias*), Pacific cod (*Gadus macrocephalus*), and walleye pollock (*Theragra chalcogramma*). Predation mortality was a flexible function of predator and prey abundances that was fitted to stomach-content data. A proof of concept illustration is presented here, assessing model outputs against a set of single-species models. The MSASA model was able to successfully estimate predation between species and integrate it into total mortality. Significant predation occurred on younger pollock and flounder. Results indicate a significant change in predation over time on pollock as a function of increased arrowtooth flounder abundance. Estimating mortality and other parameters for three species simultaneously is complex, and the advantage of greater biological realism of MSASA comes at the expense of greater uncertainty in parameter estimation.

Résumé : La prédation représente la plus importante source de mortalité pour les poissons marins et peut être un mécanisme majeur du contrôle de la taille des populations. Des modèles démographiques récents ont essayé de mesurer la prédation séparément des autres sources de mortalité naturelle. Nous basant sur ces travaux, nous avons mis au point un modèle d'évaluation plurispécifique structuré d'après l'âge (« MSASA ») pour le golfe de l'Alaska qui inclut la plie à grande bouche (*Atheresthes stomias*), la morue du Pacifique (*Gadus macrocephalus*) et la goberge de l'Alaska (*Theragra chalcogramma*). La mortalité due à la prédation est une fonction flexible des abondances de prédateurs et de proies ajustée aux données des contenus stomacaux. Nous faisons ici une démonstration de faisabilité en évaluant les données de sortie du modèle par comparaison à celles d'une série de modèles monospécifiques. Le modèle MSASA réussit à estimer la prédation des différentes espèces et à l'intégrer dans la mortalité totale. Il existe une prédation significative des goberges et plies plus jeunes. Les données indiquent un changement significatif de la prédation des goberges dans le temps en fonction de l'accroissement de l'abondance des plies à grande bouche. L'estimation simultanée de la mortalité et des autres variables chez trois espèces est complexe et l'avantage d'un plus grand réalisme biologique apporté par MSASA se fait au coût d'une incertitude accrue dans l'estimation des variables.

[Traduit par la Rédaction]

Introduction

Stock assessments have generally been written as single-species models in which many of the variables affecting abundance, whether biological or physical, have been subsumed into mortality and recruitment parameters (Quinn and Deriso 1999), and ecosystem processes have often been assumed stationary (Sainsbury 1998). These assumptions and simplifications have constrained the ability of fisheries scientists to accurately assess statistical uncertainties and can be seen as primarily due to the complexity of natural systems and secondarily due to limited computational resources. Attempts to estimate critical time-varying parameters such as natural mortality and gear selectivity have often been unsuccessful until relatively recently (Hollowed et al. 2000*a*; Kinzey and Punt 2009), and even so, many stock assessment models continue to use time-invariant inputs for such parameters for reasons of stability and parsimony (Moustahfid et al. 2009; Thompson et al. 2008). Increased understanding of the importance of these ecosystem dynamics, along with vastly expanded computing ability and the attendant push from management and research agencies to perform ecosystem-based management (Marasco et al. 2007), has resulted in a more detailed set of variables in stock-assessment models (Link 2002).

A major issue in the efforts to relax traditional stock assessment assumptions has been the replacement of a constant natural mortality with an age- and (or) time-specific

Received 10 April 2009. Accepted 19 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 22 June 2010.
J21157

Paper handled by Associate Editor Yong Chen.

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Can. J. Fish. Aquat. Sci. 67: 1135–1148 (2010) doi:10.1139/F10-053 Published by NRC Research Press

natural mortality (Fu et al. 2001). Traditional single-species stock assessment models include predation mortality under the umbrella of natural mortality, with total mortality being the sum of fishing mortality and natural mortality only. Separating predation from other sources of natural mortality should reduce the residual natural mortality coefficient along with the bias associated with assuming a constant natural mortality. Improvements in function, however, must be sufficient to offset the associated increase in the number of estimated parameters.

Andersen and Ursin (1977) developed an intricate and seminal age-structured multispecies model of the North Sea that modeled predation as a function of several factors, including size preference, prey vulnerability, predator feeding behavior, and abundance. The complexity of their model necessitated simplifications for application, and even after simplification, formal statistical estimation of parameters was not possible. However, their equations formed the basis for work by Pope (1979), Helgason and Gislason (1979), and Sparre (1980), who modified them to convert single-species virtual population analysis (VPA) into multispecies virtual population analysis (MSVPA), with predation estimated from observed stomach contents. MSVPA has remained an important tool for predation assessment within a multispecies context. MSVPA was first applied to the North Sea fish community (Pope 1991), whereas more recently, Livingston and Jurado-Molina (2000) and Jurado-Molina et al. (2005) used MSVPA to assess predation among multiple predator–prey combinations in the Bering Sea. Stomach-content data, however, are highly variable, and inferences based upon them contain substantial uncertainty that cannot be assessed through MSVPA methods, as MSVPA assumes the data to be measured without error. The same also applies to the MSVPA assumption that catch at age is measured without error.

Predation analysis has also been successfully applied within single-species, age-structured contexts. Hollowed et al. (2000*b*) modeled the effects of predation on pollock from Steller sea lion (*Eumetopias jubatus*) in the Gulf of Alaska; Livingston and Methot (1998) quantified predation on pollock from Pacific cod, northern fur seal (*Callorhinus ursinus*), and adult walleye pollock in the Bering Sea; Moustahfid et al. (2009) modeled predation on Atlantic mackerel (*Scomber scombrus*) by multiple species of demersal fish in the Northwest Atlantic. All of these studies pattern predation after fishing mortality as a product of input predator abundance and a proportionality constant. This approach, while pragmatic, is unable to model changes in predator abundance as a consequence of predator–prey interactions and does not encompass the common situation in which a species is both predator and prey. The erroneous assumption that stomach-content data are measured without error is ignored in the models.

A multispecies approach has generally been shown to have better statistical fit to data, improve estimates of mortality and recruitment, and provide a better framework for analysis of predation than single-species models (Hollowed et al. 2000*a*; Tsou and Collie 2001). Given their complexity, however, multispecies models have not yet replaced single-species models but are sometimes used in an auxiliary capacity (Dorn et al. 2008; Turnock and Wilderbuer 2007). Ecosystem models (e.g., Christensen et al. 2000) aid in defining trophic flows and connections between species, avoiding the constant natural mortality (M) assumptions in single-species assessments. Their complexity, however, does not allow for detailed assessment of predation on specific cohorts. MSVPA functions in a similar capacity, outlining the role of predation on M from an age-structured perspective instead of a mass-balanced approach.

We present a proof-of-concept description of a multispecies age-structured assessment (MSASA) model that improves upon MSVPA by using maximum likelihood methods to estimate stomach contents, as well as catch and survey components, thus allowing for error in all of those measurements. This formulation results in the partitioning of a constant natural mortality (M) into predation mortality (P) and a residual natural mortality term (M^r). The model is applied to three species in the Gulf of Alaska (GOA) with important predator–prey interactions: walleye pollock (*Theragra chalcogramma*), arrowtooth flounder (*Atheresthes stomias*), and Pacific cod (*Gadus macrocephalus*). Walleye pollock is a major prey species of both cod and flounder and together with cod constitutes the majority of the commercial fishery in the GOA. Arrowtooth flounder is a highly aggressive predator whose abundance in the GOA has sharply increased since the early 1980s, with potential effects on both cod and pollock stocks. Pacific cod is widely distributed throughout the GOA, Bering Sea, and Aleutian Islands, preying heavily on pollock and moderately on flounder.

Materials and methods

Our illustration makes a number of simplifying assumptions that enable a more direct assessment of model uncertainty arising primarily from the predation function itself, isolated from other standard sources of stock assessment variability. Output from single-species models built on these assumptions is compared with stock assessments from the Alaska Fisheries Science Center (AFSC, National Marine Fisheries Service, Seattle, Washington) to show that these constraints do not result in large deviations from accepted stock assessment values. The single-species models are then connected via predation links in a number of different configurations to identify the approach producing the best model fit to the available data and to examine changes to population dynamics arising from the estimation of predation mortality. Although current single-species stock assessments are more complex than our illustration, we posit that this complexity should present no significant obstacle to the inclusion of the MSASA predation structure and that such a process will be comparable with the transition from a series of single-species models to a single multispecies predation model as presented here.

Data and assumptions

Data common to both the single-species (SS) and multispecies (MSASA) models consist of total annual catch, total annual survey abundance, and length- and age-composition data from both the commercial catch and the survey, taken from the 2005 Stock Assessment and Fishery Evaluation (SAFE) reports (Dorn et al. 2005; Thompson and Dorn

Table 1. Summary of commercial fishery and survey data sets from the 2005 SAFE.

Data	Years available	SAFE Table
Flounder total annual catch	1981–2001	5.1 (Turnock et al. 2005)
Cod total annual catch	1981–2001	2.2 (Thompson and Dorn 2005)
Pollock total annual catch	1981–2001	1.1 (Dorn et al. 2005)
Flounder survey biomass	1984, 1987, 1990, 1993, 1996, 1999, 2001	5.3 (Turnock et al. 2005)
Cod survey biomass	1984, 1987, 1990, 1993, 1996, 1999, 2001	2.12 (Thompson and Dorn 2005)
Pollock survey biomass	1984, 1987, 1990, 1993, 1996, 1999, 2001	1.6 (Dorn et al. 2005)
Flounder survey at age	1984, 1987, 1990, 1993, 1996, 1999, 2001	5.5 (Turnock et al. 2005)
Cod survey at length	1984, 1987, 1990, 1993, 1996, 1999, 2001	2.10 (Thompson and Dorn 2005)
Pollock survey at age	1984, 1987, 1990, 1993, 1996, 1999, 2001	1.7 (Dorn et al. 2005)
Cod commercial catch at length	1981–2001	2.7*a*–2.9*b* (Thompson and Dorn 2005)
Pollock commercial catch at age	1981–2001	1.4 (Dorn et al. 2005)

2005; Turnock et al. 2005) for the years 1981–2001 (Table 1). These components are assumed measured with error and are included in the objective function. MSASA models also include annual stomach-content data, assumed measured with error, in the objective function.

Commercial fisheries are modeled by a single asymptotic selectivity curve for each species in which selectivity for ages 5+ is set to 1. For cod, this curve is an aggregate of the three gear types: pot, longline, and trawl. The majority of the pollock catch is from midwater trawls; the fishery for flounder uses bottom trawls and is limited by halibut bycatch. No length- and age-composition data exist for the flounder commercial fishery; selectivity schedules for stock assessments are estimated from bycatch, with the ASFC estimating time-invariant selectivity curves for males and females separately. As MSASA does not include bycatch, the ASFC selectivity values are used as known values to calculate a single gender-weighted selectivity curve, which was input into the model.

Survey abundance for all three species is taken from National Marine Fisheries Service (NMFS) triennial trawl surveys. Survey abundance is assumed to be proportional to total abundance as modified by the estimated survey selectivity pattern and area-swept methods.

Our assumptions aim to reduce model complexity without affecting the estimation of predation by fixing as time invariant those processes and parameters that are potentially temporally variable but appear to be extraneous to the proof-of-concept illustration. We assume the following four quantities to be temporally constant. (*i*) Length and weight at age: although some changes in length and weight at age have been observed in the modeled species over the target time period, the total variability was considered insufficient relative to the variability of the stomach-content data to justify the additional model components. (*ii*) Gear selectivity: it was beyond the scope of this study to examine temporal changes in selectivity along with the addition of predation; this would be more relevant to the actual model used for stock assessment or to future research. The use of a temporally constant selectivity curve may result in a loss of realism to a certain degree but is justified given the focus of the current paper and the complexity of the issue, which remains under discussion by current stock assessment modelers. The 2008 GOA Pacific cod stock assessment, for example, specifically addresses the difficulty in reconciling the belief that temporal variability exists in gear selectivities with the need for a parsimonious model structure (Thompson et al. 2008). As with length and weight at age, there is evidence for changes in selectivity, but it does not appear to be of a sufficient magnitude to be considered here, and the trade-off between the loss of that level of detail for ease of model assessment is considered acceptable for our purposes. (*iii*) Survey catchability (Q): the AFSC has conducted numerous studies on gear selectivity, used in calculating area-swept estimates, but many other factors that may affect catchability have not been studied. Stock assessment authors often attempt to estimate catchability in stock assessment models, but results are usually uncertain enough that it gets set to 1. Thus, fixing Q to estimate predation parameters is a valid approach that allows for improved assessment of a dynamic total mortality with no loss of the functionality of many current stock assessments. For pollock, setting $Q = 1$ is the standard base model (Dorn et al. 2008, p. 69); values for Q have often been set to 1 in cod assessments (Thompson et al. 2008, pp. 179 and 182), and the arrowtooth flounder assessment also sets $Q = 1$ (Turnock and Wilderbuer 2007, p. 453). Values for Q are therefore set to 1 for all species. (*iv*) Annual predator ingestion rate: the assumption that length and weight at age are constant implies that ingestion rate is also constant. Annual ingestion rates at age were provided by Sarah Gaichas (AFSC, Seattle, Washington) following Aydin et al. (2005) and Essington et al. (2001). Essington et al. used the allometric relationship between growth, consumption, and elimination–respiration, as expressed in the von Bertalanffy growth function for each species, to calculate ingestion rates (expressed as unit consumed per unit of predator biomass). Aydin et al. modified Essington et al.'s work to construct generalized equations for consumption in North Pacific species through prey meta-analysis. Weight-at-age data are readily available for many species, making this widely applicable. Ingestion rates were converted to an annual ingestion amount by multiplying the consumption rate per kilogram of predator by the weight at age for each species.

The AFSC Resource Ecology and Ecosystem Modeling (REEM) database provided stomach-content data taken from NMFS research trawls between 50 and 60.25°N and 169.82 and 132.88° W within the GOA from 1981–2001. No stomach data were available from 1982–1985 or from 1988–1989. Prey without ages, derived either from length or otolith readings, were not included in the analysis, and it was assumed that lack of age was not a function of age and

Table 2. Core population dynamics equations for age-structured model.

Equation	Description
$N_{i,a+1,t+1} = N_{i,a,t}\,e^{-Z_{i,a,t}}$	Abundance of species *i* at age *a* + 1 in year *t* + 1
$N_{i,a+1,t+1} = N_{i,a,t}\,e^{-Z_{i,a,t}} + N_{i,a+1,t}\,e^{-Z_{i,a+1,t}}$	Abundance in plus group of species *i* at age *a* + 1 in year *t* + 1
$F_{i,a,t} = s_{f_{i,a}} F_{i,t}$	Fishing mortality of species *i* at age *a* in year *t*
$C_{i,a,t} = \frac{F_{i,a,t}}{Z_{i,a,t}} e^{-Z_{i,a,t}} N_{i,a,t}$	Commercial catch at age of species *i* at age *a* in year *t*
$S_{i,a,t} = Q_i\, s_{s_{i,a}} N_{i,a,t}$	Survey abundance at age of species *i* at age *a* in year *t*

Table 3. Free parameters (p), inputs (i), and estimated variables (e) for models.

Variable	Description	
$N_{i,a,1}$	Initial abundance for species *i*, age *a*, in year 1 (millions of individuals)	p
$N_{i,1,t}$	Recruitment for species *i*, age 1, in year *t* (millions of individuals)	p
$F_{i,t}$	Full-recruitment fishing mortality for species *i* in year *t* (instantaneous, with units year^{-1})	p
$s_{f_{i,a}}$	Fishery selectivity for species *i*, age *a*, constant over time (proportion between 0 and 1)	
$M_{i,a,t}$ and $M'_{i,a,t}$	Total or residual natural mortality for species *i*, age *a*, in year *t* (instantaneous, with units year^{-1})	i
$P_{i,a,t}$	Predation mortality for species *i*, age *a*, in year *t* (instantaneous, with units year^{-1})	e
$Z_{i,a,t}$	Total mortality for species *i*, age *a*, in year *t*; for the SS, $Z = M + F$; for the MSASA, $Z = M' + F + P$ (instantaneous, with units year^{-1})	e
Q_i	Survey catchability (set to 1 for all species)	i
$s_{s_{i,a}}$	Survey gear selectivity for species *i*, age *a*; estimated for ages 1–4 for cod and pollock, ages 1–5 for flounder; selectivity for cod and pollock, ages 5–8, and flounder, ages 6–12, set to 1; single coefficient estimated for each species for all ages greater than those for which selectivity is set to 1 (proportion between 0 and 1)	p
$w_{i,a}$	Mean weight at age for species *i*, age *a* (kilograms)	i
$\varphi_{i,a,j,b,t}/\varphi_{j,b,t}$	Proportion by weight of prey *i,a* consumed by predator *j,b* in year *t* (proportion between 0 and 1)	e
$I_{j,b}$	Annual ingestion rate for predator *j*, age *b* (kilograms per year)	i

size or species of either predator or prey. Data were grouped within each year by predator species, predator age–length, prey species, and prey age–length. Annual stomach-content values for predator at age reflected the proportion by prey-at-age weight relative to total aggregate stomach weight summed over all predators at age.

A single predator was considered a sample of one, regardless of the number of prey items contained in its stomach. The total sample size was 2704, reflecting only those predators with stomach contents of one or more of the target prey species. Cod was the apex model predator, feeding on both flounder and pollock, with no cannibalism. Flounder fed on pollock and also exhibited some cannibalism, and pollock fed only on pollock. Predation linkages other than these were not included in the model due to low frequencies of occurrence in the stomach-content data.

Model structure

Standard equations of single-species stock assessment models (Quinn and Deriso 1999) are used to model year-class propagation, commercial catch at age, and fishing mortality (Tables 2, 3). Age cohorts begin at 1 and end in plus cohorts of 15+, 12+, and 10+ for flounder, cod, and pollock, respectively. All models follow a forward progression of cohort abundance from age 1 to the plus age group. Recruitment at age 1 is a model parameter, where annual recruitment is expressed as a deviation from an overall mean. Full-recruitment fishing mortality is defined as those ages for which gear selectivity is equal to 1 and is denoted as *F*.

Lengths and weights at age for flounder were estimated as a weighted mean between males and females for each age, as males exhibit higher natural mortality and a shorter life span than females and constitute roughly only 30% of the overall population (Turnock et al. 2005). A sex-specific model to deal with gender demographics was beyond the scope of this study.

Predation mortality

We define the instantaneous rate of annual predation mortality as the ratio of biomass of prey species *i*, age *a* (henceforth denoted *i,a*), consumed by all predators in a given year *t* relative to the total average biomass of prey *i,a* in year *t* as

$$(1)\qquad P_{\mathrm{ann},a,t} = \frac{1}{\overline{B}_{i,a,t}} \sum_{j} \sum_{b} I_{j,b} \overline{N}_{j,b,t} \frac{\varphi_{i,a,j,b,t}}{\varphi_{j,b,t}}$$

in which $I_{j,b}$ is the annual ration for a given predator of species *j*, age *b* (henceforth denoted *j,b*), $\overline{N}_{j,b,t}$ is the mean abundance of predator *j,b* in year *t*, and $\overline{B}_{i,a,t}$ is the mean

Table 4. Objective function components.

Description	Equation
Annual catch	$\sum_i\sum_t\left[\ln(C_{\mathrm{tot}_{i,t}}+0.001)-\ln(\widehat{C}_{\mathrm{tot}_{i,t}}+0.001)\right]^2$
Annual survey abundance	$\sum_i\sum_t\left[\ln(S_{\mathrm{tot}_{i,t}}+0.001)-\ln(\widehat{S}_{\mathrm{tot}_{i,t}}+0.001)\right]^2$
Catch at age	$-\sum_i\sum_a\sum_t\left[\left(\frac{C_{i,a,t}}{C_{\mathrm{tot}_{i,t}}}+0.0001\right)\cdot\ln\left(\frac{\widehat{C}_{i,a,t}}{\widehat{C}_{\mathrm{tot}_{i,t}}}+0.0001\right)\right]$
Survey abundance at age	$-\sum_i\sum_a\sum_t\left[\left(\frac{S_{i,a,t}}{S_{\mathrm{tot}_{i,t}}}+0.0001\right)\cdot\ln\left(\frac{\widehat{S}_{i,a,t}}{\widehat{S}_{\mathrm{tot}_{i,t}}}+0.0001\right)\right]$
Stomach contents	$\sum_i\sum_a\sum_j\sum_b\sum_t\left[\left(\frac{\varphi_{i,a,j,b,t}}{\varphi_{j,b,t}}+0.0001\right)-\left(\frac{\widehat{\varphi}_{i,a,j,b,t}}{\widehat{\varphi}_{j,b,t}}+0.0001\right)\right]^2$

Note: A caret denotes a quantity estimated by the model.

biomass of prey species *i,a* in year *t*. [This is analogous to the Baranov catch equation $C = F\overline{N}$.] The definition of *P* in eq. 1, however, is recursive in that predation mortality, as an element of total mortality *Z*, is also present in the calculation of $\overline{B}_{i,a,t}$. Without *Z*, mean prey abundance $\overline{N}_{i,a,t}$ and mean prey biomass $\overline{B}_{i,a,t}$ cannot be calculated. Following Lewy and Vinther (2004), we therefore approximate the true instantaneous rate of predation mortality as

$$(2)\qquad P_{i,a,t}=\frac{1}{B_{i,a,t}}\sum_j\sum_b I_{j,b}N_{j,b,t}\frac{\varphi_{i,a,j,b,t}}{\varphi_{j,b,t}}$$

using abundance and biomass of predators and prey at the beginning of each year.

The ratio $\varphi_{i,a,j,b}/\varphi_{j,b,t}$ is the proportion of prey *i,a* in all food available to predator *j,b* in year *t*, which is assumed equal to the proportion of food within the stomach of predator *j,b* in year *t* composed of prey *i,a*. Availability is the product of a suitability coefficient *v* and prey biomass:

$$(3)\qquad \varphi_{i,a,j,b,t}=v_{i,a,j,b}B_{i,a,t}$$

Suitability for modeled prey is the product of the size and species preferences of predator *j,b* for prey *i,a*, as it is assumed that size preference is independent of species preference:

$$(4)\qquad v_{i,a,j,b}=\rho_{i,j}g_{i,a,j,b}$$

in which $\rho_{i,j}$ is the species preference coefficient of predator species *j* for prey species *i*, and $g_{i,a,j,b}$ is the size-preference function. As in MSVPA, we assume that size and species of prey are the primary criteria driving prey selection by predators. The species preference coefficient accounts for the spatial overlap of predators and prey (e.g., in the water column) and the feeding mode of the predator.

Size preference is modeled as a lognormal function of the ratio between predator and prey weights following Andersen and Ursin (1977):

$$(5)\qquad g_{i,a,j,b}=\exp\left[-\frac{1}{2\sigma_j^2}\left(\ln\frac{w_{j,b}}{w_{i,a}}-\eta_j\right)^2\right]$$

in which σ and η are size-preference parameters specific to each predator *j*. A given predator *j,b* will have the same size-preference coefficient for all prey of a given size regardless of species, but *g* carries the subscript for both species and age as different prey species differ in size at age.

The total food available to a given predator *j,b* in the GOA includes many species beyond those explicitly defined in the model. To ensure that the stomach quotient (eq. 2) above correctly reflects this diet diversity, the divisor contains a nonmodeled prey component in addition to modeled prey as

$$(6)\qquad \varphi_{j,b,t}=B_{\mathrm{oth}}+\sum_i\sum_a v_{i,a,j,b,t}B_{i,a,t}$$

in which B_{oth} refers to the biomass of the nonmodeled prey, constant over time and across species and age. As this biomass is constant (set to e^{15} through trial and error), its suitability coefficient is fixed at 1, allowing size- and species-preference parameters for modeled species to be estimated relative to other prey.

AD Model Builder (ADMB, Otter Research Ltd. 2004) was used to produce maximum likelihood estimates of parameters through minimization of the objective function values (negative log-likelihood). Variances were calculated from ADMB's variance–covariance matrix. Parameter estimates were constrained where necessary; parameter bounds were set to be large enough to contain values considered to be biologically realistic but small enough to be computationally efficient. Objective function equations (Table 4) and weightings for species-specific components are provided (Table 5).

Total annual catch and total annual survey abundance were assumed to have lognormal distributions, and the weightings used were proportional to the inverse of the squared lognormal parameter σ as $1/(2\sigma^2)$, where $\sigma \approx$ coefficient of variation (CV) = standard deviation (SD)/mean. Where no such estimates of σ were available, σ values of 0.1 (weight = 50) for total annual catch and 0.2 (weight = 12) for total annual survey were used as recommended by J. Ianelli (Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington, personal communication, 2009). Catch and survey age composition were modeled as multinomial distributions, and it was assumed that the minimum effective sample size was less than the observed sample size, with a weight of 100 used for all multi-

Table 5. Akaike information criterion (AIC) values, parameter weightings (wt), and contributions to objective function scores.

	Model			
	SS (AIC = N/A)	M2 (AIC = 2156.78)	M3 (AIC = 2121.68)	M4 (AIC = 2050.65)
Total annual catch				
Flounder (wt: 50)	0.004	0.0008	0.0007	0.003
Cod (wt: 50)	0.662	0.629	0.561	0.767
Pollock (wt: 200)	0.095	0.125	0.067	0.049
Survey abundance				
Flounder (wt: 70)	15.114	13.430	12.044	11.213
Cod (wt: 19)	20.313	23.542	20.505	20.948
Pollock (wt: 12.5)	6.033	21.556	8.987	11.679
Catch at age				
Cod (wt: 100)	156.506	155.899	156.888	155.497
Pollock (wt: 100)	231.286	207.075	201.733	212.475
Survey catch at age				
Flounder (wt: 100)	63.904	57.996	38.841	37.38
Cod (wt: 100)	88.267	88.672	88.728	88.869
Pollock (wt: 100)	178.547	83.812	68.031	72.208
Stomach contents				
Period 1 (wt: 11)	N/A	35.247	25.542	21.129
Period 2 (wt: 26)	N/A	65.862	63.328	61.253
Period 3 (wt: 44)	N/A	119.539	160.581	104.848

Note: N/A, not applicable.

Table 6. Model configurations.

	Model				
	SS	M1	M2	M3	M4
M or M^r values					
Flounder age 1	0.45	0.2	0.2	0.2	0.2
Flounder age 2	0.4	0.2	0.2	0.2	0.2
Flounder ages 3+	0.245	0.2	0.2	0.2	0.2
Cod — all ages	0.37	0.37	0.37	0.37	0.37
Pollock age 1	0.9	0.2	0.2	0.2	0.2
Pollock age 2	0.8	0.2	0.2	0.2	0.2
Pollock ages 3+	0.3	0.2	0.2	0.2	0.2
Size preference	N/A	Single set	Single set	Single set	Three sets
Species preference	N/A	Single set	Single set	Three sets	Three sets
Stomach contents	N/A	Single set	Three sets	Three sets	Three sets

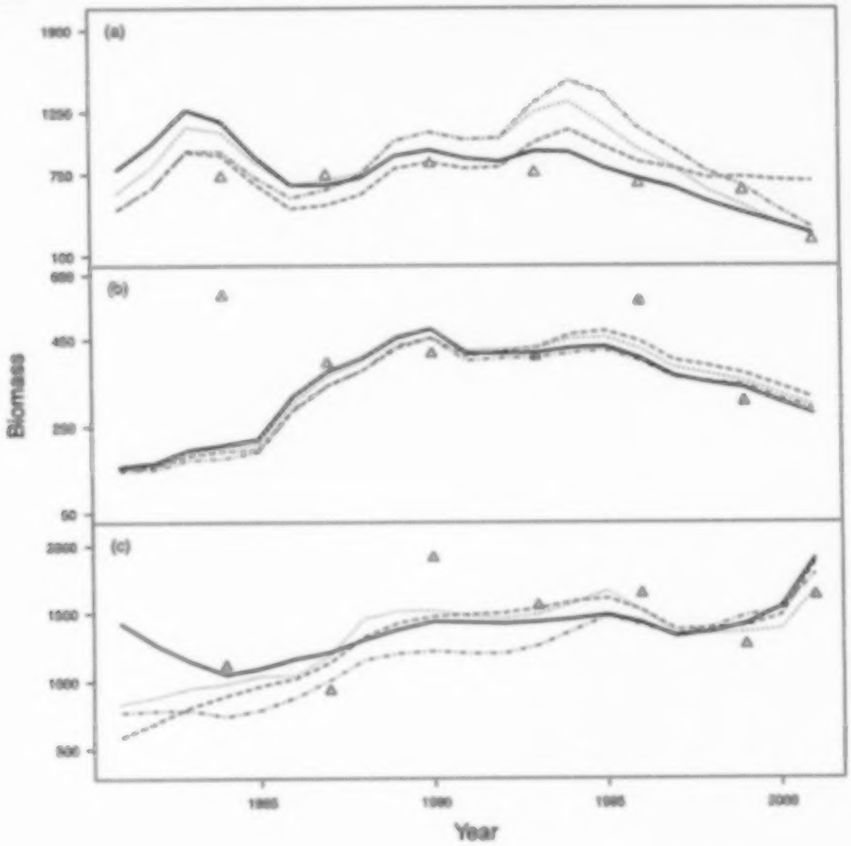
Note: N/A, not applicable.

nomials. This value is well below the limit of 400 recommended by Fournier and Archibald (1982) to avoid overweighting but sufficient to provide adequate forcing during parameter estimation. For simplicity, we assume stomach contents to follow a normal distribution, with the weightings for the stomach components of the objective function chosen empirically and set to the square root of the sample sizes for consistency after Hanselman et al. (2008), allowing the data to influence parameter estimates related to consumption.

Model configurations

Four configurations of size- and species-preference parameters were evaluated (Table 6). As above, observed stomach-content data had been grouped within each year by predator species, predator age and length, prey species, and prey age and length; stomach-content values for each predator *j,b* reflected the annual mean prey-at-age proportions by prey-at-age weight relative to total stomach weight over all predators *j,b*. The first MSASA model (M1) estimated a time invariant set of size- and species-preference parameters and was fitted to a mean over all years of annual mean predator-at-age stomach content values. The second MSASA model (M2) also estimated a single set of size- and species-preference parameters but was fitted to stomach-content data that were divided into three blocks in which each set of annual stomach proportions was averaged over 7 years (1981–1987, 1988–1994, 1995–2001). The third MSASA model (M3) was fitted to the 7-year averaged values of the stom-

Fig. 1. Estimated survey biomass for (*a*) pollock, (*b*) cod, and (*c*) flounder from each model configuration compared with biomass values from the triennial surveys (expressed as tonnes): SAFE biomass values, triangles; SS, continuous line; M2, broken line; M3, dotted line; M4, dotted–dashed line.



ach data and estimated a single set of size-preference parameters but a set of species-preference parameters for each averaging period. The fourth MSASA model (M4) modified the M3 approach to include estimation of separate size-preference parameters for each averaging period.

Natural mortality values (*M*) for the single-species (SS) models were taken from the 2005 SAFE, with higher rates for prey species ages 1 and 2 to mimic predation. Residual natural mortality values (M^r) for MSASA configurations were set to a single value for all ages of a given species (Table 6).

The AIC was used to compare model results (Burnham and Anderson 2002) from models M2–M4, which used the same data. Variance of parameter estimates was also compared between models. The AIC was calculated as $-2\ln L + 2p$, in which p is the number of estimated parameters (Table 6).

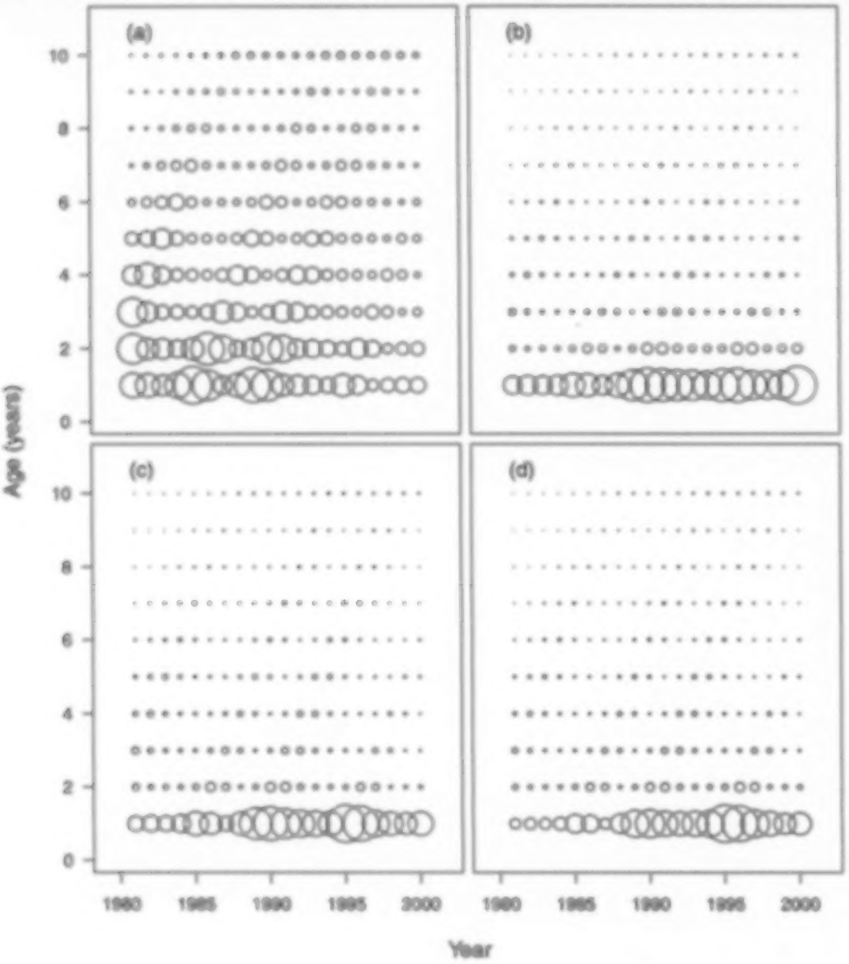
Results

The SS models matched the general SAFE biomass trends for all three species (Fig. 1), with the exception of cod biomass in the 1984 survey. Estimates of total annual catch matched the SAFE data with almost no deviation (not shown). We conclude that fixing some of the potentially time-varying parameters to temporally constant forms as discussed above did not adversely affect model output and that the assessment of predation built on that construct is most likely valid.

Model M1 was removed from consideration because AD Model Builder was unable to estimate the variance–covariance matrix for that configuration without excessively increasing the weighting values on the stomach components in the objective function. Although this weighting allowed the estimation of variance, it also resulted in poorer fits of other model parameters due to overweighting of the stomach components, an effect that has been noted in other multispecies work (Kinzey and Punt 2009).

The remaining MSASA model estimates of biomass closely matched the SS estimates (Fig. 1). The largest divergence was pollock biomass in the mid-1990s in model M4,

Fig. 2. Estimated pollock abundance by age and year for (*a*) the single-species model, (*b*) model M2, (*c*) model M3, and (*d*) model M4, with the area of a given circle denoting abundance (scale differs between subplots). Note the increase in pollock biomass in the 1990s for the multispecies models, which reflects the additional biomass required to support the higher predation during that period.



which reflects increases in predation on pollock in the multispecies model. MSASA estimates of flounder biomass in the early 1980s were somewhat lower than those of the SS model. As cod had no modeled predators, biomass varied little between any model configuration. Total annual catch for all species showed little variation from SS values (not shown). Pollock abundance at age 1 increased compared with the SS (Fig. 2), and flounder population trends followed a similar pattern (not shown). Cod abundance, as with biomass, showed little variation between models (Fig. 3).

General predation curves for pollock followed reasonable and expected trends (Fig. 4), with the highest levels on age-1 pollock and the divisions between time periods clearly visible in the results from model M4. Predation on age-1 pollock by other pollock ranged from 0.55 to 2.98, predation from flounder on age-1 pollock was 0.29–1.6 and from cod was 0.02–0.41. Predation from flounder rose steadily over time, reflecting the attendant increase in flounder abundance, whereas predation levels from cod remained relatively stable except in model M2, which showed an increase over time. Predation on flounder varied among models more so than pollock (Fig. 5). The highest predation was on age-1 flounder for the M2 and M3 models, whereas the M4 model showed highest predation on age-1 flounder only in period 1. The M3 and M4 models also showed a drastic increase in overall predation on flounder in periods 2 and 3 relative to period 1. Graphical residual analysis produced no patterns indicative of model bias (not shown). Cannibalism in pollock was the major source of age-1 mortality but was not present in older age classes, whereas cannibalism in flounder was proportionally less but distributed across ages 1–6 (not shown).

The M4 model had the lowest AIC value, followed by M3 and then M2 (Table 5). Despite differences in specific pa-

Fig. 3. Estimated cod abundance by age and year for (*a*) the single-species model, (*b*) model M2, (*c*) model M3, and (*d*) model M4, with the area of a given circle denoting abundance (scale differs between subplots).

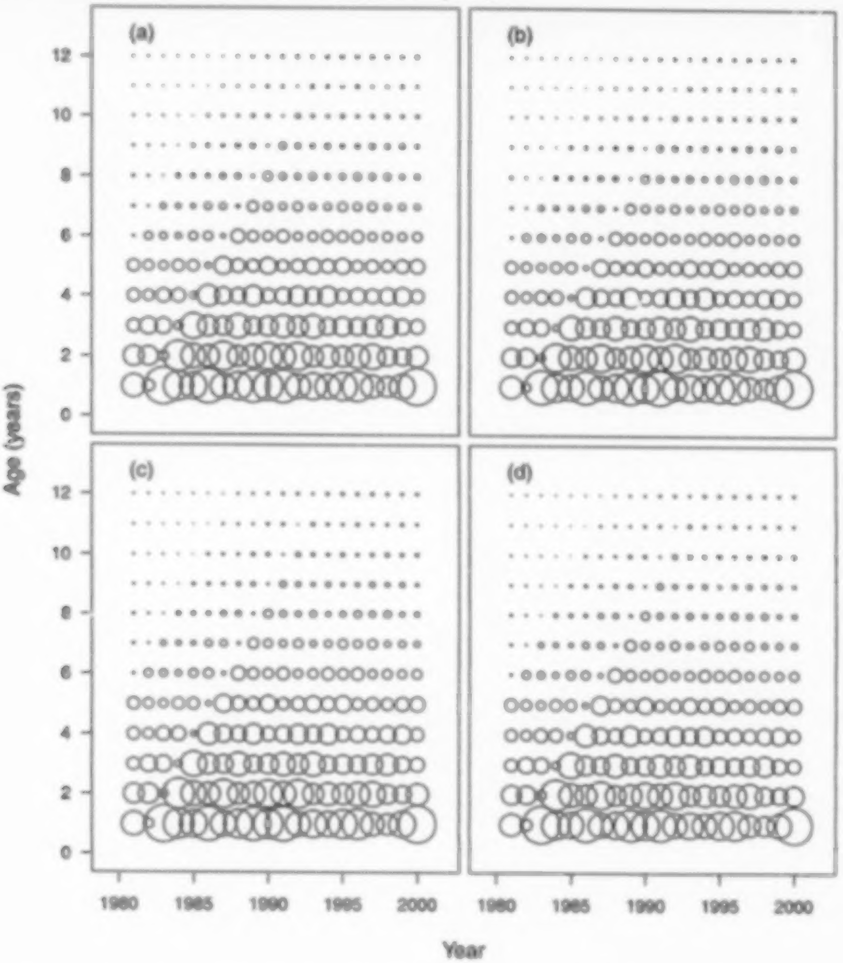
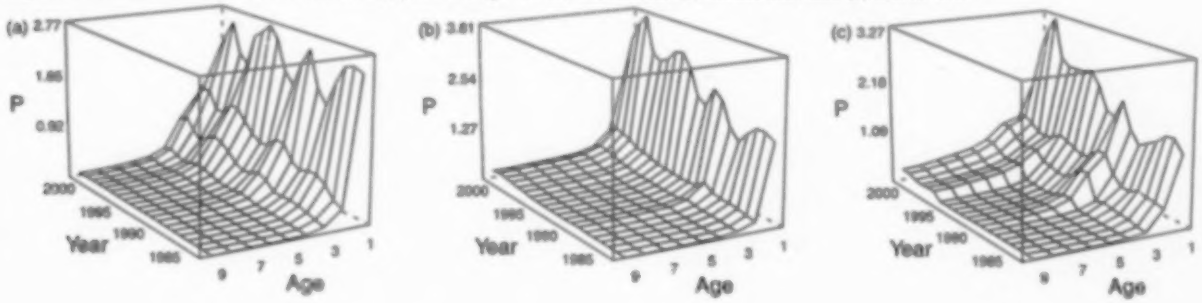


Fig. 4. Predation mortality (*P*) for pollock by year and age for (*a*) model M2, (*b*) model M3, and (*c*) model M4.



rameter estimates and variances, the estimates of abundance, predation, and catch between models M2, M3, and M4 were very similar. The largest objective function values were from commercial fishery catch-at-age components, followed by survey catch at age; the smallest were from estimates of total annual catch, in which all component values were less than 1.

Size-preference curves (eq. 5) showed the expected log-normal function relative to increase in predator age and size

Fig. 5. Predation mortality (*P*) for flounder by year and age for (*a*) model M2, (*b*) model M3, and (*c*) model M4.

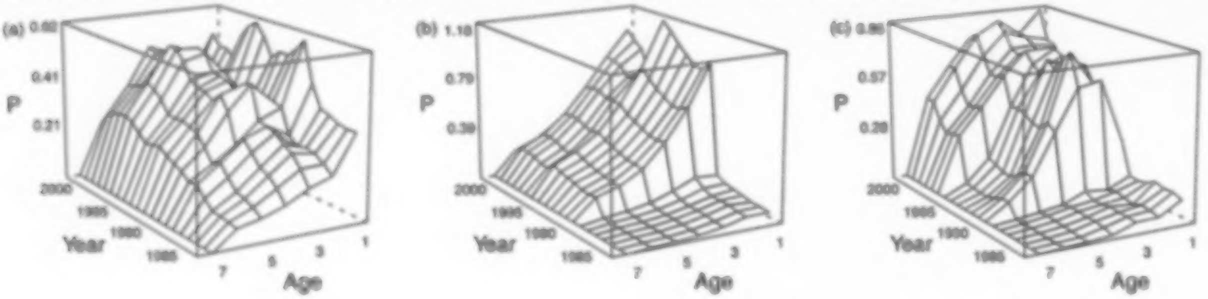


Fig. 6. Size-preference curves (eq. 5) from model M3 for cod, age 6 (continuous line), age 9 (broken line), and age 12 (dotted line), graphed against log prey weight (in kilograms).

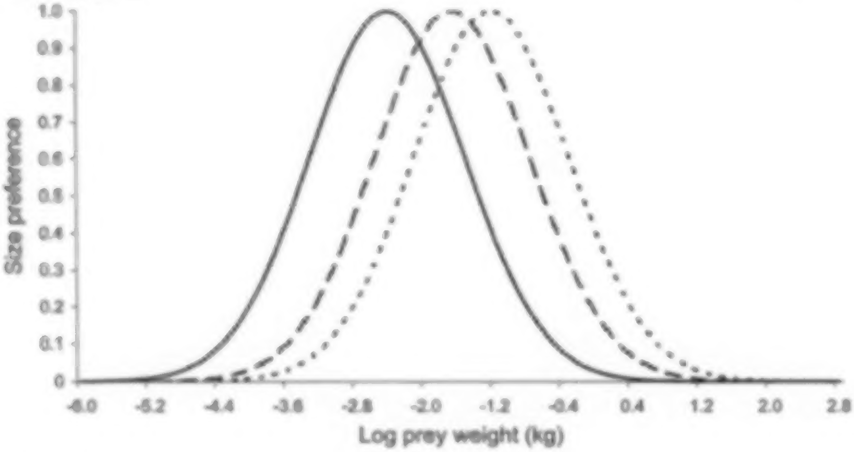
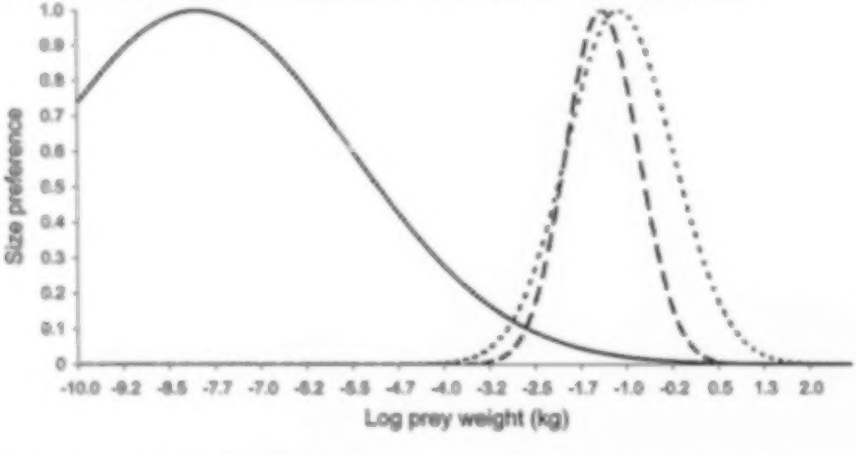


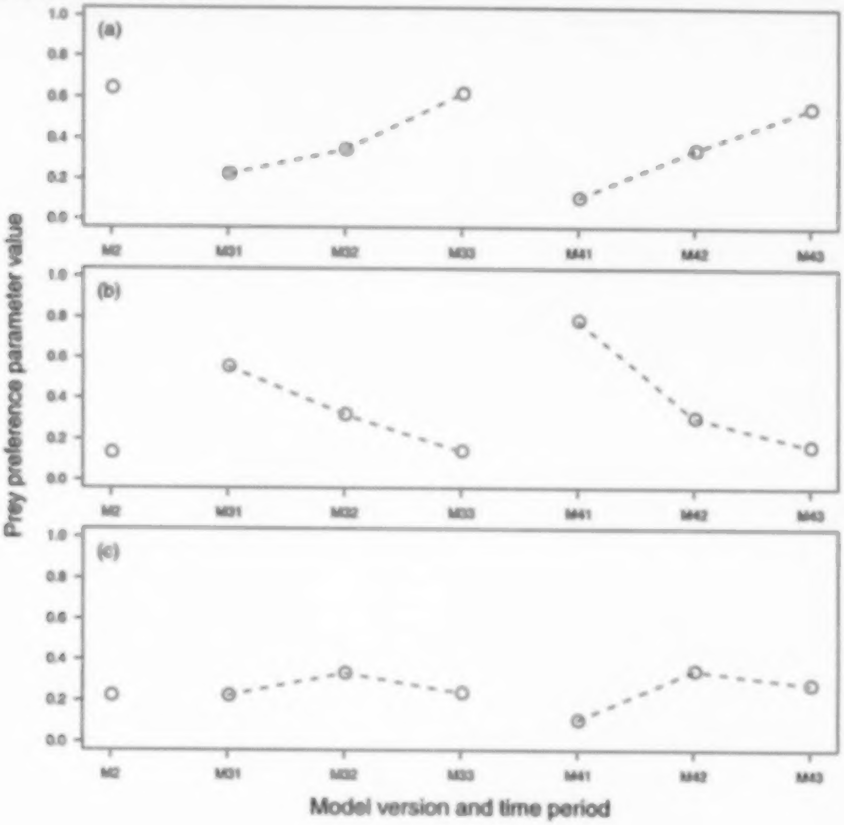
Fig. 7. Size-preference curves (eq. 5) from model M4 for age-15 flounder period 1 (continuous line), period 2 (broken line), and period 3 (dotted line) showing changes in size preference over time, graphed against log prey weight (in kilograms).



(Fig. 6), but varied over time for a given predator, especially in period 1 (Fig. 7). The corresponding coefficients of variation (estimated from the delta method) had a wide range, with the mean CVs for estimates of η (M2 = 0.115, M3 = 0.041, M4 = 0.023) lower than those for σ (M2 = 0.343, M3 = 0.137, M4 = 0.628).

Estimates of species-preference parameter values (ρ) were similar between the M3 and M4 models (Fig. 8), and changes in prey preferences between periods followed similar trends. Variability for these parameters was lowest for the M3 model overall, although no particular predator–prey combination displayed consistently higher or lower variances across model con-

Fig. 8. Species preference (ρ) for cod preferring (*a*) flounder, (*b*) pollock, and (*c*) other food over all model configurations and time periods (scaled to sum to one for each model configuration).



figurations. Within the M3 and M4 models, the first two time periods showed higher variability than period 3.

Alternative model configurations

In an attempt to relax assumptions regarding survey catchability (Q) and natural mortality (M or M^r), efforts were made to include these parameters in estimation procedures. Estimability of these parameters was evaluated by ADMB outputs, including maximum gradient components, standard deviations, and a positive definite Hessian matrix, as well as biological realism and data fits, as per Merritt and Quinn (2000).

Attempts to include Q as an estimated parameter in both the singe-species model and the MSASA models were inconclusive. The initial response of all models to predation was to increase abundance, which lowered Q in many instances to unrealistic values, with estimates of parameter variances unable to be calculated.

When natural mortality was estimated along with Q, values for M or M^r increased, contrary to the expectation that values would decline due to predation. This increase may have been a response to the greatly increased abundance necessary to meet the demands of predation on younger age classes, but which then resulted in abundances of older ages that were too large. Setting bounds for natural mortality had the same effect as with Q. Although some models produced reasonable values for M^r when Q was set to 1 for all species, this result was not consistent or reproducible over all model configurations and seemed dependent on extreme weightings for stomach components in the objective function.

It would appear that neither Q nor M (or M^r) is freely estimable under the current model structure and available data. This is in keeping with stock assessment methods of attempting to estimate these quantities externally and inputting the results into assessment models and reflects the general difficulty involved in the estimation of natural mortality (Quinn and Deriso 1999).

An attempt was also made to estimate parameters through a MSASA model that made no use of averaging when fitting to the stomach-content data, but instead used the available data from each individual year. Where no data were available, no objective component for that year was included. Size- and species-preference parameters were single sets, as in M1 or M2. This configuration did not converge, regardless of varying approaches to objective function weighting schemes or parameter bounds.

Discussion

In estimating predation, MSASA was able to extract the signal emanating from statistically imperfect stomach-content data and produce measures of predation generally in keeping with other multispecies models with respect to higher predation levels on younger prey cohorts, increased abundance when predation was modeled relative to single-species models, and temporal variability in predation levels. MSASA estimates of predation mortality on age-1 pollock ranged from 1.37 to 3.43. Hollowed et al. (2000*a*) found predation mortality in the GOA on age-1 pollock to fall between 0.34 and 0.8. These estimates are lower than in the current study, but Hollowed et al.'s work did not consider cannibalism as a major source of predation mortality due to low rates of cannibalism during diet sampling seasons, whereas MSASA shows cannibalism to be the main source of predation on age-1 pollock. Overall estimates of total natural mortality ($M' + P$) for age-1 pollock in Hollowed et al.'s work (0.85–2.0), were also lower than MSASA estimates (1.57–3.63). In the Bering Sea where cannibalism is recognized as a major factor in the dynamics of pollock stocks, the upper ranges of predation mortality estimates for age-1 pollock approach MSASA estimates: 1.1–1.9 (Livingston 1993) and 0.2–1.6 (Livingston and Methot 1998). Livingston and Jurado-Molina (2000) included additional predator species relative to the 1998 study and found mean predation mortality for age-1 pollock to be 1.85.

Differential digestion rates relative to prey size may play a role in diet data variability. Digestion is generally assumed to proceed more rapidly on prey surfaces that are directly in contact with the stomach lining (K. Aydin and T. Buckley, Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington, personal communication, 2009). Thus, single large prey items digest at a rate roughly equivalent to a bolus of smaller prey, and smaller fish are more likely to be consumed in multiple quantities. Digestion is also assumed to be exponential, with relatively little bias between large and small prey. As both bony structures and otoliths are used in aging (pollock aging often uses otolith methods if skeletal structures have been compromised), the potential bias in aging smaller prey items is further reduced. Simulations have shown that for fish larger than 40–50 mm in length, little correction for digestion rates is needed in the modeled species (K. Aydin and T. Buckley, AFSC-NMFS, personal communication, 2009), and it therefore seems unlikely that differing digestion rates contribute much to data variability.

Although both prey species exhibited increased abundance at age 1, biomass did not show an attendant increase, with the exception of pollock in the M4 configuration. The implication is that although recruitment increases to meet the needs of predation, the increased biomass is consumed within the first year, and in contrast with other predation studies, this increased abundance in prey species is not reflected in older cohorts. Although the reason remains unclear, it is potentially due to the fact that MSASA models predation in a multispecies context. Predation in a single-species context can increase prey biomass to meet predation demands with no feedback effect, whereas a multispecies approach requires a balance between predation between species that can act as both predator and prey upon each other. The fact that the apex predator cod did not display a reduction in older cohorts offers possible support to the idea. An alternate explanation is that the current models do not include predators of sizes large enough to exert major predation pressure on older cohorts. Hollowed et al. (2000*a*) found that larger predators such as Steller sea lion and Pacific halibut preferred larger prey, feeding on ages that had already entered the commercial fishery and thus requiring an increased abundance at older ages. With the addition of larger predators, prey abundances in MSASA applications may also display increased abundance in older cohorts, and therefore increased overall biomass, relative to single-species studies (Plagányi 2007).

Data from the pollock triennial survey indicate that although overall pollock abundance is declining, abundance at age 1 is increasing. Yet the individual age classes from the SS model do not match this trend, showing a decline in the abundance of all ages. Individual age classes from MSASA models, however, do indeed show this increase in age-1 abundance with a decline in subsequent age classes. This indicates that the effects of predation (increased abundance in heavily predated age classes) are being echoed in the survey data, and that models that include predation match these survey indices better than SS models.

These results suggest that predation is often a hidden process, focused predominantly on younger ages (but recall the potential consequences of including larger predators in the MSASA, as discussed above), and that management procedures that primarily focus on fish that have reached the age of recruitment into the commercial fishery do not directly observe its effects. High predation rates, however, have a significant effect on recruitment into the fishery because increased predation requires increased productivity to reach the same number of fishery recruits (Collie and Gislason 2001). Catch levels that ignore high predation on early ages may have adverse effects on spawning biomass, especially when the relative abundance of predator and prey species changes over time. In addition, the overlap effect, in which the earliest cohorts entering the fishery are still being affected by the extremely high predation levels common to younger ages, has yet to be assessed (Moustahfid et al. 2009).

The result of the high variability present in the stomach data was that predation also varied widely over time. This variation reflects both real changes in feeding and predation over time and data that are simply "noisy" due to sampling variability. A natural mortality *M* that is constant over time and age fails to capture this variability (Hollowed et al. 2000*a*; Moustahfid et al. 2009). Using a single set of species-preference parameters (M2) did not perform as well as using three sets (M3, M4), indicating that these changes in predation were partially due to changes in prey preference. However, shifts in abundance occurring on time frames shorter than the 7 years used for the averaging periods were lost by that averaging. Further exploration of the time scale over which predation and predation functional feeding are best modeled is needed to better incorporate this uncertainty into stock assessment models.

Predation is a continuous process, but the current approach models predation similar to pulse fishing, in which removals occur in toto at the beginning of each year. This approximation allows for a solution to the recursion in eq. 1 but incurs bias when predation is high (such as younger cohorts), an issue addressed but not resolved in similar predation models (Lewy and Vinther 2004). These models, like the current work, have also shown that this approximation of an instantaneous rate of predation mortality allows values for P to exceed 1, which would be expected for younger cohorts experiencing high predation mortality. The problem is that for this to happen, the consumption of prey i,a would have to exceed its biomass if predator and prey biomass were unchanging. Reduction or elimination of this bias through implementation of subannual time frames for model evaluation or development of a true instantaneous predation mortality is a high priority for model improvement.

The predation model by Hollowed et al. (2000*a*) for pollock in the GOA included predator functional responses to changes in prey abundance. Kinzey and Punt (2009) employed seven different potential functional responses in their evaluation of predation, including competition between predators. The current MSASA implementation incorporates a type-II functional relationship (Holling 1959) between predation and prey abundance such that predator satiation occurs at high prey abundance, as demonstrated in Magnússon (1995). Inclusion of additional predator behaviors and the effects on model performance should be explored as model development continues.

The lower AIC score for model M4 was unexpected. Examination of objective function values shows that the improved AIC score for model M4 relative to model M3 was due almost entirely to a marked reduction in the score for the stomach-content component in period 3 from 168.561 (M3) to 104.351 (M4). This may be due to the data for the two earlier periods being insufficient to drive better size- and prey-preference estimates, a hypothesis supported by the higher variances of size- and prey-preference parameters for those periods. Further work is needed to determine whether changes in size preference accurately reflect changes in prey cohort structure and should continue to be included.

Although model M4 performed the best of the model configurations examined, this may be due in part to the weightings applied in the objective function, as well as the variability of stomach-content data discussed above. When stomach content weights were increased, better fits to diet data and lower variances in the size- and species-preference parameters were produced, but poorer overall performance and fits to catch and survey data resulted. Better stomach data, combined with additional exploration to weighting approaches, might have resulted in a better performance by model M3, emphasizing the sensitivity of these models to data weighting and the complexity of weighting choices (Kinzey and Punt 2009). Although it appears clear that changes in species and size preference affect the estimation of predation, it remains to be seen how such shifts should best be modeled.

The changing effects of predation on abundance must be taken into account when setting harvest levels (Collie and Gislason 2001). The North Pacific Fishery Management Council's Scientific and Statistical Committee recommended in 2003 that predation from arrowtooth flounder be considered within the stock assessment for pollock in the GOA (Dorn et al. 2005). It also addressed the difficulty of assessing predation mortality in setting catch levels, indicating that the assumption of a constant natural mortality that contains predation is insufficient for future fisheries management purposes. Our illustration has shown that multispecies age-structured framework is a promising approach to modeling predation and has much to offer in the transition from single-species approaches to models that better incorporate ecosystem considerations. By using maximum likelihood to estimate diet parameter values, we were able to reduce the bias inherent in use of a constant natural mortality term in proportion to the number of predators included in the model structure. The decision to use a single-species or multispecies approach, however, depends on researcher objectives, and the trade-off must be made between the advantage of MSASA information and greater biological realism versus the disadvantage of increased uncertainty due to additional model complexity and the high variability of stomach-content data. Further work with simulations of known populations and alternative parameterizations, including different size-preference distributions and the inclusion of predator functional responses, are the next steps for development of the MSASA approach.

Acknowledgements

This publication is the result of research sponsored by Alaska Sea Grant with funds provided by the National Oceanic and Atmospheric Administration Office of Sea Grant, Department of Commerce, under grant no. NA 16RG2321 (project no. R/31-11), and from the University of Alaska with funds appropriated from the State. This publication is the result, in part, of research and education sponsored by the Cooperative Institute for Arctic Research with funds from the National Oceanic and Atmospheric Administration under cooperative agreement NA 17RJ1224 with the University of Alaska. The authors thank Steven Cadrin and an anonymous referee for their extensive and constructive reviews. The authors also thank Pat Livingston for providing the Resource Ecology and Ecosystem Modeling stomach-content data and suggestions for analyses, Chris Hay-Jahans and Pat Livingston for serving on the Master's committee for the primary author, Jim Ianelli for advice and instruction regarding ADMB code and model construction, Kiersten Curti for code testing and trouble-shooting, Andre Punt for reviewing the initial paper and providing suggestions, and Doug Kinzey for input on predation code and ADMB modifications.

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Wintertime ocean conditions synchronize rockfish growth and seabird reproduction in the central California Current ecosystem

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Abstract: Chronologies developed from annual growth-increment widths of splitnose rockfish (*Sebastes pinniger*) and yelloweye rockfish (*Sebastes ruberrimus*) otoliths were compared with time series of lay date and fledgling success for the common murre (*Uria aalge*) and Cassin's auklet (*Ptychoramphus aleuticus*) in the north-central California Current. All time series were exactly dated and spanned 1972 through 1994. In a principal components analysis, the leading principal component ($PC1_{bio}$) accounted for 64% of the variance in the data set. By entering the upwelling index, the Northern Oscillation index, sea surface temperatures, and the multivariate ENSO (El Niño Southern Oscillation) index into principal components analysis, a time series of environmental variability $PC1_{env}$ was developed for each month of the year. Over the interval 1972 through 1994, $PC1_{bio}$ most strongly correlated with $PC1_{env}$ for February and, to a lesser extent, January and March. Moreover, when each of the six biological time series was related to the 12 $PC1_{env}$ through stepwise multiple regression, February was always the most significant ($p < 0.01$). The same was true if upwelling index was substituted for $PC1_{env}$. As upper-trophic predators, rockfish and seabirds independently corroborate that wintertime ocean conditions are critical for productivity in the California Current ecosystem.

Résumé : Nous avons comparé les chronologies obtenues à partir des largeurs des incréments de croissance annuelle sur les otolithes du sébaste canari (*Sebastes pinniger*) et du sébaste aux yeux jaunes (*Sebastes ruberrimus*) et les séries chronologiques des dates de ponte et du succès de l'envol chez le guillemot marmette (*Uria aalge*) et le starique de Cassin (*Ptychoramphus aleuticus*) dans le centre nord du courant de Californie. Toutes les séries chronologiques portent des dates précises et couvrent la période de 1972 à 1994. Dans une analyse des composantes principales, la première composante principale ($PC1_{bio}$) explique 64 % de la variance de l'ensemble de données. Nous avons produit une série chronologique de la variabilité de l'environnement $PC1_{env}$ pour chaque mois de l'année en incluant l'indice de résurgence, l'indice d'oscillation boréale, les températures superficielles de la mer et l'indice multidimensionnel ENSO (oscillation australe d'El Niño) dans l'analyse des composantes principales. Dans l'intervalle de 1972 jusqu'à la fin de 1994, la $PC1_{bio}$ est le plus fortement corrélée avec $PC1_{env}$ en février et, à un degré moindre, en janvier et en mars. De plus, lorsque chacune des six séries chronologiques biologiques est mise en relation avec les 12 $PC1_{env}$ par une régression multiple pas à pas, février est toujours le mois le plus significatif ($p < 0{,}01$). Il en est de même si on substitue l'indice de résurgence à $PC1_{env}$. En tant que prédateurs de haut niveau trophique, les sébastes et les oiseaux marins confirment de manière indépendante que les conditions de l'océan en hiver sont déterminantes pour la productivité de l'écosystème du courant de la Californie.

[Traduit par la Rédaction]

Introduction

Climate variability is increasingly recognized as a major factor influencing structure, function, and productivity in the California Current ecosystem (CCE) of the Northeast Pacific (McGowan et al. 1998; Chavez et al. 2003). In the CCE, regional changes in upwelling, stratification, and temperature have been linked to productivity and reproductive success in a wide range of species. Among the most sensitive to environmental variability include several upper-trophic-level species such as the Cassin's auklet (*Ptychoramphus aleuticus*; Sydeman et al. 2006), common murre (*Uria aalge*; Miller and Sydeman 2004), shortbelly rockfish (*Sebastes jordani*; Field et al. 2007), splitnose rockfish (*Sebastes diploproa*), and yelloweye rockfish (*Sebastes ruberrimus*; Black et al. 2008; Black 2009), as well as lower-trophic-level planktonic species including copepods (Roemmich and McGowan 1995; Hooff and Peterson 2006) and

Received 16 July 2009. Accepted 20 March 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 24 June 2010.
J21306

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Can. J. Fish. Aquat. Sci. 67: 1149–1158 (2010) doi:10.1139/F10-055 Published by NRC Research Press

krill (Dorman et al. 2005). Life history traits and growth patterns of these species typically respond at annual time scales and integrate or amplify ecosystem variability and, as such, represent potentially powerful, yet underutilized, indices of ecosystem response to climate forcing.

Establishing the effects of climate on growth requires observational time series of sufficient length to ensure adequate statistical power and reasonably capture the historical range of variability inherent to the system. To date, a number of time series of direct observations exist for several fisheries of the Northeast Pacific, including such variables as catch (Spencer and Collie 1997; Hare et al. 1999; Field and Ralston 2005), recruitment (Hollowed and Wooster 1992; Hollowed et al. 2001), and body size and fecundity (Harvey et al. 2006). These data sets provide valuable insight into long-term patterns of variability and their relationships with climate. However, repeated sampling is costly and the number of lengthy time series of direct measurements is consequently limited. Apart from direct observations, various proxies of environmental variability can be used, e.g., the scales or other hard parts of fishes preserved in varved sediments (Soutar and Isaacs 1974). Another increasingly applied approach is the analysis of growth-increment widths in otoliths or other bony structures using dendrochronology (tree-ring analysis) techniques (Black et al. 2005, 2008; Black 2009). To date, several exactly dated chronologies have been developed for rockfish in the Northeast Pacific that strongly relate to climate records, corroborate indices of productivity, and thus represent a novel means by which to summarize the influences of climate on growth (Black et al. 2005, 2008; Black 2009).

In this study, we incorporate several multidecadal time series from Pacific rockfish and seabirds, all of which are centered on the Farallon Islands off the central California coast. The data consist of egg-laying dates and reproductive success (chicks per pair) for Cassin's auklet and common murre, as well as otolith growth-increment chronologies for splitnose and yelloweye rockfish. Considering the wide range of life histories and mechanisms through which lower-trophic productivity has been integrated, these time series will likely be relevant to additional species of the CCE, especially shared food sources such as krill. In addition, these biological records may be compared with climatic records to establish not only those variables to which rockfish and seabirds are most sensitive, but also the times of year (i.e., seasons) in which these sensitivities are greatest. An increasing number of studies suggest that January, February, and March ocean conditions are critical for CCE productivity (Logerwell et al. 2003; Miller and Sydeman 2004; Schroeder et al. 2009), even though the upwelling processes traditionally associated with productivity are largely spring, summer, and autumn phenomena (Bograd et al. 2009). Thus, the objectives of this study are (*i*) to explore the interrelationships among rockfish and seabird time series in the CCE, (*ii*) to extract shared patterns of variability to generate multispecies indicators, and (*iii*) to relate time series and their derivatives to climate data, with particular emphasis on better establishing the importance of wintertime ocean variability to these diverse measures of biological function in the CCE.

Materials and methods

Biological time series

Six biological time series were used in this study: (*i*) lay date for Cassin's auklet, (*ii*) lay date for common murre, (*iii*) breeding success for Cassin's auklet, (*iv*) breeding success for common murre, (*v*) an otolith growth-increment chronology for splitnose rockfish, and (*vi*) an otolith growth-increment chronology for yelloweye rockfish. These six time series were selected because of their length, spatial overlap, temporal overlap, and the fact that all time series were exactly dated and contain measures of success or failure specific to each calendar year.

For both Cassin's auklet and common murre, phenology and breeding success data have been collected at the Farallon Islands (located approximately 30 miles west of San Francisco Bay, California, USA; 37.7°N and –123.0°W) by Point Reyes Bird Observatory under contract with the US Fish and Wildlife Service since 1972 (Ainley et al. 1995; Sydeman et al. 2001; Schroeder et al. 2009). During the breeding season (March–July), individual pairs of auklets and murres were selected and studied for dates of egg laying and overall annual reproductive output. Auklet pairs were observed every five days beginning on 2 March, whereas murres were studied every day from 16 April to ~1 July. Date of egg laying (reported as day of the year from 1 to 365) was defined as the date when an egg was first noted for each pair, and breeding success was defined as the proportion of pairs successfully rearing a chick to independence (for auklets), or to "jumping" stage (for murres). Murre offspring leave the breeding colony when development is incomplete (approximately half grown), so unlike the auklets, the breeding success of murres is defined as survival of the chicks to a departure stage, which is typically when they are 20–25 days old (Sydeman 1999). In the case of auklets, pairs may attempt to raise a second chick within a single year, though success is extremely rare. Notwithstanding these unusual occurrences, the parameter "breeding success" of these seabirds is binary, fledged or failed (0 or 1), as they lay only a single egg per reproductive season.

Cassin's auklets are planktivorous and feed on krill, whereas the diet of common murres is more varied and consists of krill, Pacific hake, market squid, juvenile rockfish, anchovy, and sardines (Sydeman et al. 1997, 2001; Mills et al. 2007). As for the rockfish, yelloweye consume juvenile rockfish, herring, sandlance, and flatfishes, as well as shrimps and crabs, whereas splitnose feed primarily on krill (Love et al. 2002). The yelloweye and splitnose rockfish chronologies were developed using annual growth-increment widths from otoliths obtained by commercial and sport fishing vessels and NOAA National Marine Fisheries Service surveys. Though exact locations for several samples are unknown, all otoliths were collected over the continental shelf between approximately 35°N and 39°N (Black et al. 2005, 2008). For details on otolith preparation and development of these splitnose and yelloweye rockfish chronologies, see Black et al. (2005, 2008). In summary, otoliths were embedded in resin, mounted on a lapidary saw, and thin-sectioned through the dorsal–ventral axis to 0.4 mm in thickness. Otolith thin sections were polished with 2000-grit sandpaper and 10 µm lapping film to reveal the annual increments, which were best viewed with reflected light in splitnose rockfish and transmitted light in yelloweye rockfish. Within each species, samples were visually crossdated to ensure that all annual growth increments were assigned the correct calendar year. Universally applied in tree-ring studies, crossdating is the process of matching among all specimens in a sample set the synchronous growth patterns in increment width induced by climate. If an annual increment has been missed or falsely added, the synchronous growth pattern will be offset by a year relative to that in the other samples, and the point at which the offset begins indicates the location of the error. Without crossdating, errors can accumulate and diminish accuracy, especially in early portions of a chronology. A total of 48 otoliths were used to develop the splitnose rockfish master chronology and 27 were used to develop the yelloweye rockfish master chronology.

After visual crossdating, the dorsal half of the otolith was then photographed and the annual increment widths were measured continuously from the margin to as close to the focus as possible using the program ImagePro Plus (version 6.0, Media Cybernetics, Bethesda, Maryland). Crossdating was statistically verified with the Dendrochronology Program Library program COFECHA (Holmes 1983; Grissino-Mayer 2001; Black et al. 2005). Each otolith measurement time series was then fit with a negative exponential function and divided by the values predicted, thereby removing age-related growth declines and standardizing each time series to a mean of 1. Within each species, these detrended time series were averaged to form the master chronology in which each value represented sample-wide growth for a given calendar year. A value greater than 1 represented above-average growth, whereas a value below 1 represented below-average growth. All chronology development was conducted using the program ARSTAN (developed by Ed Cook and Paul Krusic, available at http://www.ldeo.columbia.edu/res/fac/trl/public/publicSoftware.html). The quality of each chronology was quantified using the expressed population signal (EPS) statistic, which describes how well the sample means represents the mean of the theoretical population from which it was drawn. Though there is no significant threshold for this statistic, an EPS value of 0.85 or greater is considered adequate (Wigley et al. 1984).

Physical time series

Monthly averages of four environmental variables including sea surface temperatures (SST), upwelling (UW), the Northern Oscillation index (NOI), and the multivariate ENSO index (MEI) were related to the time series of rockfish growth and seabird reproductive success. Sea surface temperatures were obtained from the International Comprehensive Ocean-Atmosphere Data Set (ICOADS) and averaged between 36°N and 40°N and from 131°W to the coast, a geographic extent comparable with that of rockfish sampling. The coastal upwelling index (UI) represents the magnitude of the offshore component of Ekman transport (Bakun 1973; Schwing et al. 1996). Data from the upwelling station located at 39°N were used in this study. The NOI is the anomaly sea level pressure difference between the annual mean position of the North Pacific High and Darwin, Australia, and captures the strength of atmospheric circulation between the tropics and the North Pacific, particularly with respect to the El Niño – Southern Oscillation (Schwing et al. 2002). NOI (1948–2007) and UW (1946–2007) records were obtained through the Pacific Fisheries Environmental Laboratories live-access server at http://www.pfeg.noaa.gov/. Finally, the MEI is the leading principal component of six marine and atmospheric variables in the tropical Pacific and was obtained (1950–2005) from the NOAA Earth Systems Research Laboratory (http://www.cdc.noaa.gov/people/klaus.wolter/MEI/). These four variables represent a combination of local (SST, UW) and regional (MEI, NOI) indices that have been shown to relate to the biology of the California Current ecosystem (Roemmich and McGowan 1995; Miller and Sydeman 2004; Dorman et al. 2005). All biological and environmental variables included the years 1972 through 1994, and the analysis in this study was consequently restricted to this common interval.

Detrending and normalization

The detrending procedure used to generate the rockfish growth-increment chronologies would have eliminated any long-term trends, if present. To ensure equal treatment of the data, long-term trends were removed from all biological and environmental time series over the common interval of 1972–1994. Each biological and environmental time series was fit with a linear regression, and where significant ($p < 0.05$), the observed values were divided by the predicted values. Cassin's auklet lay date was the only biological or environmental time series in which a regression was significant ($p < 0.05$). Finally, each environmental and biological time series was normalized to a mean of 0 and standard deviation of 1.

Relationships among biological and physical time series

Principal components analysis was used to develop a single environmental time series for each month of the year using the monthly resolved MEI, NOI, UW, and SST data. For each of the 12 months of the year, a 4 × 23 data matrix was constructed with one column for each of four environmental variables and one row for each year over the 1972–1994 common interval. Thus, each cell was the monthly mean of MEI, NOI, UW, or SST for a given calendar year. The leading principal component ($PC1_{env}$) was extracted from each of the 12 data matrices, capturing between 44.8% and 60.1% (mean = 52.1%) of the variance. In so doing, 48 environmental variables were distilled into 12.

A principal components analysis was performed that included all six biological time series over the common interval of 1972–1994. More specifically, a 6 × 23 data matrix was constructed with one column for each biological time series and one row for each calendar year spanned by the analysis. The leading principal component ($PC1_{bio}$) was used as an index of shared variability among the rockfish and seabird data sets. Next, the 12 monthly environmental principal components ($PC1_{env}$) were correlated with the six biological time series, as well as the leading principal component ($PC1_{bio}$) from the biological time series. To summarize relationships among the biological and environmental time series, a redundancy analysis (RDA) was performed that included the six biological time series and the 12 monthly $PC1_{env}$ as environmental variables (Legendre and Legendre 1998). RDA is a canonical form of principal com-

Table 1. Pairwise correlation coefficients (r) among bird and rockfish time series, as well as correlations (loadings) with the leading principal component ($\mathrm{PC1}_{\mathrm{bio}}$) extracted from the six time series, all over the common interval of 1972–1994.

	Span	Cassin's auklet lay date	Common murre success	Cassin's auklet success	Yelloweye rockfish	Splitnose rockfish	$\mathrm{PC1}_{\mathrm{bio}}$	First-order autocorrelation
Common murre lay date	1972–2006	**0.94**	**-0.93**	**-0.64**	–0.38	**-0.71**	-0.95	0.08
Cassin's auklet lay date	1972–2006	1.00	**-0.90**	**-0.62**	–0.41	**-0.66**	-0.93	0.05
Common murre success	1972–2006		1.00	**0.67**	**0.46**	**0.66**	0.95	–0.11
Cassin's auklet success	1972–2006			1.00	0.26	**0.45**	0.74	0.01
Yelloweye rockfish chronology	1948–2001				1.00	0.23	0.49	**-0.52**
Splitnose rockfish chronology	1948–1994					1.00	0.80	–0.12

Note: Cassin's auklet, *Ptychoramphus aleuticus*; common murre, *Uria aalge*; splitnose rockfish, *Sebastes diploproa*; yelloweye rockfish, *Sebastes ruberrimus*. Significant ($p < 0.05$) correlations are in bold, with the exception of $\mathrm{PC1}_{\mathrm{bio}}$, which is a function of the biological time series. Lay dates negatively correlate with the other time series, indicating that high fledgling success and early lay dates, both indicators of reproductive success, are associated with above-average rockfish growth. Also shown is the first-order autocorrelation for each time series.

ponents analysis in which axes were restricted to linear combinations of the environmental variables. Finally, stepwise multiple regressions ($p < 0.01$ to enter) were performed to better identify the months of the year during which environmental variability was most closely linked to rockfish growth and seabird reproduction. A separate regression was performed on each biological time series, including ($\mathrm{PC1}_{\mathrm{bio}}$), with 12 monthly $\mathrm{PC1}_{\mathrm{env}}$ from the current year plus five monthly $\mathrm{PC1}_{\mathrm{env}}$ from the previous year (August, September, October, November, and December lagged by one year) as predictors. The procedure was then repeated with upwelling, a variable more easily interpreted than $\mathrm{PC1}_{\mathrm{env}}$ and likely the most relevant of the original four environmental indices (UW, NOI, MEI, SST) considered in this study.

The stability of the regression between $\mathrm{PC1}_{\mathrm{bio}}$ and the 12 $\mathrm{PC1}_{\mathrm{env}}$ was assessed using a bootstrapping procedure. At each iteration ($n = 1000$) of the bootstrapping technique, a calibration data set equal to the number of years shared by both variables ($n = 23$) was randomly selected with replacement. Thus, some years in this shared time period were omitted, while other years were selected multiple times. Those years not selected were used as an independent verification data set. The reduction of error coefficient (RE), a measure of shared variance between the independent data and the values modeled from the calibration data set, was also calculated at each iteration (Fritts 1976). The maximum theoretical value of the RE statistic is +1, and a positive value indicates that the regression model has some degree of skill.

As a way to summarize the physical forcings on the biological variables, composite maps of wind and sea level pressure (SLP) were constructed from the five highest and lowest years of $\mathrm{PC1}_{\mathrm{bio}}$. The wind and SLP data used for the composite maps were from the National Centers for Environmental Prediction (NCEP) North American Regional Reanalysis (NARR), with data starting in 1979 for the region spanning 33°N to 55°N and 140°W to 120°W.

Results

All biological time series exceeded 30 years in length and overlapped by a minimum of 23 with a common interval of 1972 through 1994 (Table 1), and the EPS for both rockfish chronologies exceeded 0.85 during that time period (data not shown). The yelloweye rockfish chronology contained significant ($p < 0.05$) autocorrelation, and effective sample size was adjusted according to the methods of Pyper and Peterman (1998) prior to correlation analyses. The majority of time series significantly ($p < 0.05$) correlated with one another, and in general, seabird lay date and the rockfish chronologies were positively related, yet negatively related to seabird reproductive success (Table 1). Thus, early lay date and high fledgling survival, both indicators of reproductive success, were positively associated with one another, as well as with rockfish otolith growth. All nonsignificant relationships involved the yelloweye rockfish chronology (Table 1).

The leading principal component of the six biological time series ($\mathrm{PC1}_{\mathrm{bio}}$) calculated across the common interval of 1972 to 1994 explained 69% of the variance in the data set (eigenvalue of 4.1) and significantly loaded against all six measurement time series (Table 1). $\mathrm{PC1}_{\mathrm{bio}}$ separated lay dates from reproductive success and the rockfish chronologies, negatively loading against the two lay date time series

Fig. 1. Principal component scores of six biological time series centered on the Farallon Islands, central California: a yelloweye rockfish (*Sebastes ruberrimus*) otolith growth-increment chronology, a splitnose rockfish (*Sebastes diploproa*) otolith growth-increment chronology, mean lay date for common murre (*Uria aalge*) and Cassin's auklet (*Ptychoramphus aleuticus*), and fledgling success for common murre and Cassin's auklet. The common period shared by all time series is 1972 to 1994.

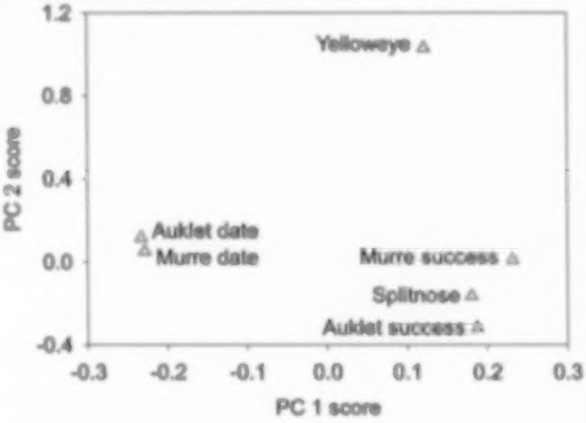
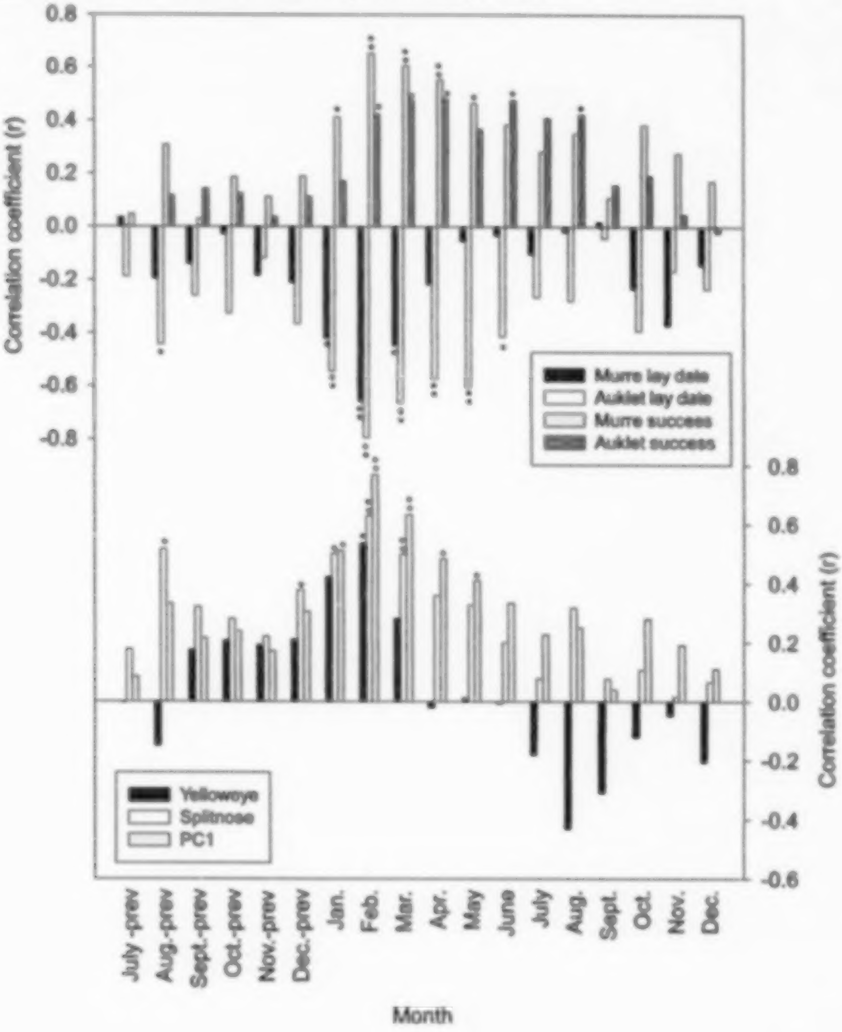


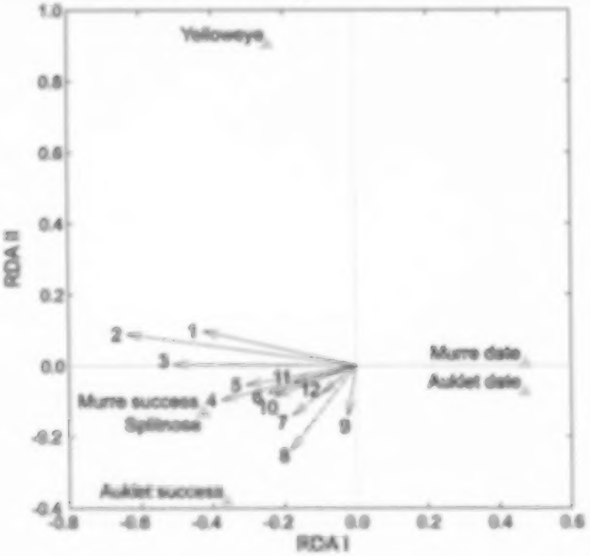
Fig. 2. Correlations between a monthly resolved time series of climate and the time series of seabird reproduction and rockfish growth. The climate variable is the leading principal component from upwelling, sea surface temperature, the Northern Oscillation index, and the multivariate ENSO index ($PC1_{env}$) for each month of the current year (lag 0), as well as the prior year (prev) (lag –1) through July. The biological time series are as follows: a yelloweye rockfish (*Sebastes ruberrimus*) otolith growth-increment chronology, a splitnose rockfish (*Sebastes diploproa*) otolith growth-increment chronology, mean lay dates for common murre (*Uria aalge*) and Cassin's auklet (*Ptychoramphus aleuticus*), and fledgling success for common murre and Cassin's auklet. In addition, the leading principal component ($PC1_{bio}$) from these six biological time series is correlated with climate. *, $p > 0.05$; **, $p > 0.01$.



and positively loading against the other four time series (Table 1; Fig. 1). Overall, positive values of $PC1_{bio}$ indicated years of favorable seabird reproductive success (early lay dates and fledgling success) and vigorous rockfish otolith growth. The second principal component explained an additional 14% of the variance, with an eigenvalue of 0.86. This component significantly ($p < 0.05$) loaded only against the yelloweye chronology, separating it from the other five time series (Table 1; Fig. 1). Because of the low portion of variance captured and the fact that it was only related to one of the six time series, the second principal component was dropped from further analysis. Removing linear trend in the seabird time series minimally affected the analysis, and $PC1_{bio}$ calculated prior to detrending related very strongly to that calculated after detrending ($R^2 = 0.997$). No linear trends were identified in the environmental data.

For each month of the year, the leading environmental principal component ($PC1_{env}$) positively loaded against UW and NOI and negatively loaded against SST and MEI such that high values of each $PC1_{env}$ reflected cool ocean conditions, while low values of each $PC1_{env}$ reflected warm ocean conditions. In summary, the correlations between the $PC1_{env}$ and the biological time series indicated that cool ocean conditions were associated with rapid rockfish otolith growth

Fig. 3. Redundancy analysis (RDA) of the six biological time series and 12 monthly PC$_{\text{env}}$. In this biplot, each triangle corresponds to a biological time series (common murre (*Uria aalge*) lay date, Cassin's auklet (*Ptychoramphus aleuticus*) lay date, common murre fledgling success, Cassin's auklet fledgling success, splitnose rockfish (*Sebastes diploproa*) otolith growth-increment chronology, and yelloweye rockfish (*Sebastes ruberrimus*) otolith growth-increment chronology), and each arrow represents one of the 12 monthly environmental variables (1 = January PC$_{\text{env}}$, …, 12 = December PC$_{\text{env}}$). February PC$_{\text{env}}$ (arrow 2) is the most important of the environmental variables and is strongly correlated with axis I.



and seabird reproductive success in the form of early egg lay dates and increased fledgling survival (Fig. 2).

Correlations with environmental variables tended to be highest from January through April, especially for murre lay date, murre success, auklet lay date, and the splitnose rockfish chronology (Fig. 2). An exception was auklet success, which maintained nearly continuous correlations with PC1$_{\text{env}}$ from February through June and July (Fig. 2). Also anomalous, though not significant, were negative correlation between the yelloweye rockfish chronology and PC1$_{\text{env}}$ in late summer (Fig. 2). The leading principal component of the six biological time series (PC1$_{\text{bio}}$) captured the strongest climate–growth relationships of any of the six original time series in the study. PC1$_{\text{bio}}$ positively loaded against the rockfish chronologies and seabird fledgling success while negatively loading against seabird lay date such that positive values indicated years with favorable rockfish growth and seabird reproduction. Positive correlations between PC1$_{\text{bio}}$ and PC1$_{\text{env}}$ reflected that good years for rockfish and seabirds were associated with cool, upwelling-favorable ocean conditions (Table 1; Fig. 2).

In the RDA, RDA I accounted for 54% of the variance in the data set and separated seabird lay date from fledgling success and the rockfish chronologies, similar to the pattern observed in the PCA (Fig. 3). With respect to environmental variables, RDA I was most strongly correlated to the winter months, especially January ($r = -0.43$), February ($r = -0.64$), and March ($r = -0.51$) PC$_{\text{env}}$ (Fig. 3). RDA II accounted for only 7% of the variance in the data set and separated the yelloweye rockfish chronology from the other biological time series. This secondary axis was most strongly correlated with late summer and early autumn months, including July ($r = -0.14$), August ($r = -0.24$), and September ($r = -0.14$) PC$_{\text{env}}$. Overall, the RDA closely followed the PCA with a leading axis related to winter and a secondary axis related to summer. These axes accounted for only 10% less of the variance than did those in the PCA, indicating that the 12 PC$_{\text{env}}$ explained a large portion of the variability in the biological data.

Multiple regression analysis confirmed that February was the most important month of the year for rockfish growth and seabird reproductive success. Relationships between biological time series and PC$_{\text{env}}$ time series or UW were nonlinear, and log transformation of PC$_{\text{env}}$ and UW time series eliminated problems with autocorrelation in the regression residuals. For this reason, all PC$_{\text{env}}$ and UW time series were log-transformed prior to regression analysis. For each regression, February was the most important month for all biological time series whether PC$_{\text{env}}$ or UW were used as predictor variables (Table 2). Also, R^2 values were comparable for UW and PC$_{\text{env}}$, although summertime PC$_{\text{env}}$ were also significant for auklet fledgling success and the splitnose chronology. Signs for each regression, which were positive for all but lay date, were consistent with all other analyses in that favorable rockfish growth and seabird reproduction were associated with cool, upwelling-favorable ocean conditions.

The relationship with upwelling was heavily influenced by the two extreme years of 1992 and 1983 (Fig. 4), yet even when these values were removed, the regression was still significant ($R^2 = 0.29$, $p = 0.01$). A bootstrapping analysis confirmed that the relationship between PC1$_{\text{bio}}$ and February upwelling was stable. Mean R^2 for the dependent data sets was 0.64 (±0.01 95% confidence interval, CI), whereas mean R^2 for the independent data sets was somewhat lower, but still quite high at 0.58 (±0.02 95% CI). Mean reduction of error (RE) was strongly positive at 0.74 (±0.01 95% CI), indicating agreement between the independent data and the values modeled from the calibration data sets.

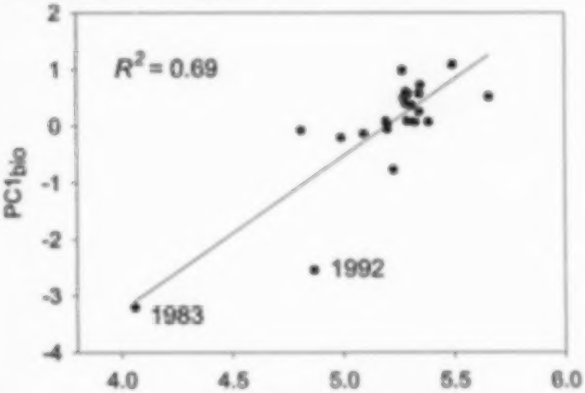
On a broader spatial scale, the timing and magnitude of coastal upwelling in the central California Current is impacted by the strength and position of the North Pacific High (NPH) sea level pressure (SLP) system. Composite maps of SLP and surface wind vectors are presented for the five years of highest PC1$_{\text{bio}}$ values (1985, 1982, 1989, 1988, 1979; Fig. 5*a*) and the five years of lowest PC1$_{\text{bio}}$ values (1983, 1992, 1986, 1990, 1993; Fig. 5*b*). In the five years with highest PC1$_{\text{bio}}$ values, the mean center of the NPH was at 35°N, 135°W and had a mean amplitude of ~1023 mbar. The 10 m wind vectors along the coast associated with this NPH pattern are directed towards the southeast (upwelling-favorable), with magnitudes exceeding 4 m·s^{-1}. The SLP composite map (Fig. 5*b*) of the five years with lowest PC1$_{\text{bio}}$ has the center of the NPH (25°N, 130°W) farther south and weaker than the five years of highest PC1$_{\text{bio}}$. North of 36°N, the mean wind vectors along the coast are directed towards the north and northeast, i.e., downwelling-favorable. Also,

Table 2. Results for multiple stepwise regressions.

Time series	Month	Sign	R^2 (p)
PC_{env}			
Cassin's auklet lay date	February	–	0.71 ($p < 0.001$)
Common murre lay date	February	–	0.64 ($p < 0.001$)
Cassin's auklet success	February	+	0.24 ($p = 0.008$)
	July	+	0.20 ($p = 0.014$)
Common murre success	February	+	0.53 ($p < 0.001$)
Splitnose rockfish chronology	February	+	0.49 ($p < 0.001$)
	August	+	0.12 ($p = 0.003$)
Yelloweye rockfish chronology	February	+	0.28 ($p < 0.001$)
$PC1_{bio}$	February	+	0.69 ($p < 0.001$)
Upwelling			
Cassin's auklet lay date	February	–	0.66 ($p < 0.001$)
Common murre lay date	February	–	0.66 ($p < 0.001$)
Cassin's auklet success	February	+	0.33 ($p = 0.004$)
Common murre success	February	+	0.57 ($p < 0.001$)
Splitnose rockfish chronology	February	+	0.43 ($p < 0.001$)
Yelloweye rockfish chronology	February	+	0.25 ($p = 0.009$)
$PC1_{bio}$	February	+	0.69 ($p < 0.001$)

Note: Cassin's auklet, *Ptychoramphus aleuticus*; common murre, *Uria aalge*; splitnose rockfish, *Sebastes diploproa*; yelloweye rockfish, *Sebastes ruberrimus*. Each biological time series was regressed against 17 environmental time series, one environmental variable for each month of the year plus five (prior August through prior December) lagged from the previous year. The analysis was performed using PC_{env} as environmental variables and then repeated using upwelling. Months that were significant ($p > 0.01$) in the regression are shown, in addition to July PC_{env} for auklet success, which was very close to the 0.01 threshold. The sign of the relationship (positive or negative), R^2, and p value are shown for each significant variable.

Fig. 4. Relationship between log-transformed February upwelling and $PC1_{bio}$. The extreme years of 1983 and 1992, which heavily influence the relationship, are shown.



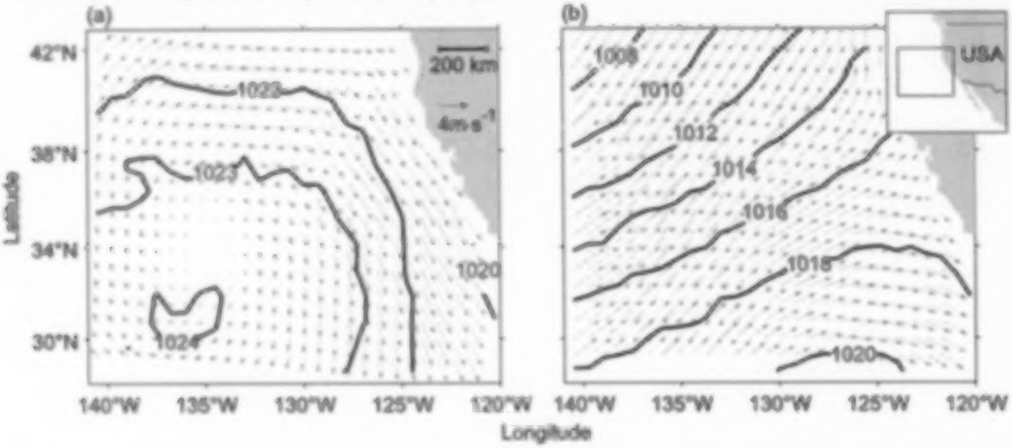
the magnitudes of the wind vectors around the Farallones are small (<2 m·s^{-1}).

Discussion

Indices of rockfish growth and seabird reproductive success are exactly dated, share a uniquely long interval of more than 20 years, and experience a high degree of covariability. Interrelationships among these diverse time series almost certainly reflect the influence of climate on lower-trophic productivity. For example, earlier research has identified strongly positive relationships between the rockfish chronologies and coastal upwelling, the principal driver of primary and secondary productivity in the CCE (Black et al. 2008; Black 2009). Such climate–growth relationships are consistent with unusually narrow growth-increment width in widow rockfish (*Sebastes entomelas*) and low levels of visceral fat in yellowtail rockfish (*Sebastes flavidus*) during the 1983 El Niño, an event characterized by abnormally warm water and below-average levels of upwelling in the CCE (Lenarz and Echeverria 1986; Woodbury 1999). Further supporting a link between fish growth and climate, Pacific salmon (*Oncorhynchus* spp.) catch tends to be higher during relatively cool, productive periods in the California Current, especially on decadal time scales (Hare and Francis 1995; Hare et al. 1999). As for seabirds, early lay date for common murres and Cassin's auklets has been linked to strong upwelling in the CCE (Schroeder et al. 2009). Moreover, Jones et al. (2008) found that Brandt's cormorant (*Phalacrocorax penicilliatus*) lay date and fledgling success in the central CCE were significantly reduced in the 1998 El Niño, particularly in comparison with the much greater upwelling and productivity that occurred during the 1999 La Niña. Overall, the time series of rockfish otolith growth and seabird reproductive success used in this study appear to be linked through a shared sensitivity to climate variability, especially upwelling.

Upwelling and the productivity that it enables are generally regarded as spring and summertime phenomena, with greatest intensities between the months of May and July (Bograd et al. 2009). However, the synchrony shared be-

Fig. 5. Composite maps of February sea level pressure (SLP) and wind vectors for (*a*) mean of the five highest $\mathrm{PC1}_{\mathrm{bio}}$ years, corresponding to the best conditions for rockfish growth and seabird reproduction, and (*b*) mean of the five lowest $\mathrm{PC1}_{\mathrm{bio}}$ years, corresponding to the poorest conditions for rockfish growth and seabird reproduction. The SLP and wind vectors are from the North American Regional Reanalysis and start in 1979. For clarity, arrows are plotted at every degree.



tween rockfish growth and seabird reproductive success is a function of shared sensitivity to wintertime, especially February, ocean variability. Indeed, other studies have addressed the importance of ocean conditions early in the year, particularly with respect to the start of the productivity season as indexed by the spring transition. In the CCE, the spring transition has been related to a wide range of ecosystem dynamics, including upwelling (Schwing et al. 2006), zooplankton diversity (Hooff and Peterson 2006), and recruitment of salmonids (Logerwell et al. 2003) and crustaceans (Shanks and Roegner 2007). The definition of spring transition varies among studies and may involve upwelling, sea level, or some combination thereof (Logerwell et al. 2003; Kosro et al. 2006; Shanks and Roegner 2007). Yet when calculated across broad regions, all indicate that the spring transition is much later in the northern CCE than in the southern CCE. The spring transition occurs, on average, on 30 January at 36°N latitude in comparison with 24 April at 45°N latitude (Bograd et al. 2009), generally consistent with a mean date of 31 March for the Oregon coast according to Logerwell et al. (2003). Thus, for our study region, the spring transition date may occur in February, though it does not relate as strongly to the rockfish and seabird time series as measures of upwelling intensity. In comparison with a spring transition index defined as the date when the cumulative upwelling index reaches its minimum value (Bograd et al. 2009), relationships with $\mathrm{PC1}_{\mathrm{bio}}$ ($R^2 = 0.39$, $n = 23$ years, $p = 0.001$) are considerably weaker than that between $\mathrm{PC1}_{\mathrm{bio}}$ and February $\mathrm{PC}_{\mathrm{upw}}$ ($R^2 = 0.59$, $n = 23$ years, $p < 0.0001$) or the log-transformed February upwelling intensity ($R^2 = 0.69$).

These relationships suggest that a specific date may not be as important as the upwelling dynamics prior to and near the beginning of the spring transition, which is February for this study region. Variation in January and February upwelling intensity relates much more strongly to $\mathrm{PC1}_{\mathrm{bio}}$ than to spring transition date. In their analyses of the auklet and murre lay date time series, Schroeder et al. (2009) suggest that anomalous wintertime upwelling preconditions the system, priming it for high levels of productivity early in the season. During the winter months prior to or near the spring transition, north winds are intermittent, though water column stratification is generally weak. Under these conditions, even relatively short-duration upwelling-favorable winds could provide pulses of nutrients to stimulate primary productivity (Schroeder et al. 2009). Consistent with this mechanism, Logerwell et al. (2003) also noted the importance of winter ocean climate in their analysis of coho salmon (*Oncorhynchus kisutch*) marine survival along the Oregon coast. Weak downwelling winds during the winter were associated with weak water column stratification in the spring, preconditioning the system for high levels of productivity (Logerwell et al. 2003).

For seabirds, the nutritional status of females is known to affect the timing of lay date (Perrins 1970; Jones et al. 2008). Along the British Columbia coast at Triangle Island, Sorensen et al. (2009) found that the prebreeding diet of Cassin's auklets with early lay dates was energetically superior to the prebreeding diet of those birds with later lay dates. Though lay date ranged from early April through mid-May, the prebreeding diet in February and March was critical not only for lay date phenology, but also for egg size. In the CCE, an increase in productivity in January, February, and March would be well timed to influence the condition of breeding females, the results of which would be realized later in the spring as eggs are laid and chicks are fledged in late March through early June (Schroeder et al. 2009). Mean lay date for Cassin's auklet was 14 April and that for common murre was 14 May, as calculated for the interval 1972 through 1994. With respect to rockfish, high levels of productivity in January, February, and March could have the effect of lengthening the growing season. Perhaps relatively low levels of upwelling later in the spring and summer are adequate for productivity, but favorable winter conditions allow growth during a period when it would not otherwise occur. The initiation of the productivity season appears to be much more important than its conclusion, as evidenced by the general lack of climate correlations in the late summer and fall.

Wintertime ocean conditions may also be important farther north, considering that rockfish chronologies in the CCE relate to rockfish and Pacific geoduck chronologies along the British Columbia coast through shared sensitivities to climate in January, February, and March (Black et al. 2008; Black 2009). Also, the biological and physical indices described in this study may be relevant to other species in the CCE. For example, $PC1_{env}$ for February significantly relates to the Sacramento River index for salmon (1983–1994, $n = 12$ years, $R^2 = 0.32$, $p = 0.05$). The leading principal component from the rockfish and seabird time series provides a closer fit (1983–1994, $n = 12$ years, $R^2 = 0.46$, $p = 0.01$), suggesting that wintertime ocean conditions are important to salmon ecology and also that the $PC1_{bio}$ may serve as a more reliable ecosystem indicator than physical variables.

In summary, rockfish and seabird time series both provide a multiproxy, multidecadal, and annually resolved "view from the top" by integrating ecosystem productivity after it has cascaded up through lower trophic levels. The fact that the data set includes species with such diverse life histories, diets, and processes through which biological success is expressed (i.e., growth and reproduction) provides strong evidence as to the importance of wintertime ocean variability. From an applied perspective, these time series indicate significant bottom-up regulation and underscore the importance of climate to ecosystem function prior to the peak upwelling season. Also, the leading principal component from these rockfish and seabird time series could represent a new multispecies indicator of regional productivity with potential relevance to ecosystem-based management. Moreover, the relationship between February upwelling and $PC1_{bio}$ reflects strongly nonlinear responses between the physical environment and ecology of the CCE such that strong climatic extremes such as the 1983 El Niño exert a disproportionately large influence on the system. Thus, February upwelling, in particular, could have predictive value as an early indicator of productivity in the coming year, and on longer time scales, forecasts of February ocean conditions could be highly relevant for assessing the biological impacts of future climate change and variability. Future work will be necessary to determine whether similar relationships between wintertime ocean conditions and rockfish growth and seabird reproduction extend beyond the 23-year window used in this study. Yet the combination of diverse species used in this study suggests that wintertime ocean conditions prior to peak upwelling are an important component of CCE structure and function.

Acknowledgements

The seabird research was conducted on the Farallon National Wildlife Refuge under contract between PRBO Conservation Science (formerly Point Reyes Bird Observatory) and the US Fish and Wildlife Service (USFWS). We thank PRBO and USFWS for data contributions to this project. Funding for this analysis and write-up was provided by NOAA's Fisheries and the Environment (FATE) program and the California Ocean Protection Council and California Sea Grant under grant R/OPCENV-07 entitled "Tackling climate change and ecological complexity: matches and mismatches in the seasonal cycle of California's marine flora and fauna". Funding was also provided through the National Science Foundation under grant 0929017 entitled "Importance of winter upwelling to California Current ecosystem dynamics." This is contribution no. 5 of the Farallon Institute and contribution no. 2510 of the Bodega Marine Laboratory.

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Abundance and distribution of Antarctic krill (*Euphausia superba*) nearshore of Cape Shirreff, Livingston Island, Antarctica, during six austral summers between 2000 and 2007

Joseph D. Warren and David A. Demer

Abstract: Abundance and distribution of Antarctic krill (*Euphausia superba*) in the nearshore waters north of Livingston Island, Antarctica, were characterized from six small-boat surveys conducted in late January or early February from 2000 to 2007. The first three surveys (2000, 2002, 2004) were conducted using a 120 kHz split-beam echosounder to measure water column acoustic backscatter. The last three surveys (2005–2007) were conducted using 38 kHz and 200 kHz single-beam echosounders. A portion of the acoustic backscatter was attributed to Antarctic krill based on the results of net tows, underwater video observations, and a multiple-frequency acoustic classification algorithm. The annual mean krill biomass density in the survey area ranged from 11 to 84 $g \cdot m^{-2}$. Results are compared with the western Scotia Sea area of the US Antarctic Marine Living Resources (AMLR) program's acoustic surveys of krill biomass density for the same years. Nearshore krill biomass densities were significantly larger (t test, $p < 0.05$), more stable, and the coefficients of variation were smaller than the much larger AMLR surveys. Increased competition between seals, penguins, and humans for the nearshore krill resource, especially during the austral summer months, could impact the recruitment success of these land-based krill predators. Implications of nearshore krill biomass on small-scale management units are discussed.

Résumé : Six inventaires en petit navire réalisés à la fin de janvier ou au début de février de 2000 à 2007 nous ont servi à caractériser l'abondance et la répartition du krill antarctique (*Euphausia superba*) dans les eaux côtières au nord de l'île Livingston, Antarctique. Les trois premiers inventaires (2000, 2002, 2004) ont été faits à l'aide d'un échosondeur de 120 kHz à faisceau divisé afin de mesurer la rétrodiffusion acoustique dans la colonne d'eau. Les trois derniers inventaires (2005–2007) ont utilisé des échosondeurs à faisceau unique de 38 kHz et de 200 kHz. Une partie de la rétrodiffusion acoustique est attribuée au krill antarctique, d'après les résultats des échantillonnages au filet et les observations à la vidéo sous-marine et à l'aide d'un algorithme de classification acoustique à fréquences multiples. La densité de biomasse annuelle moyenne du krill dans la zone d'échantillonnage varie de 11 à 84 $g \cdot m^{-2}$. Nous comparons nos résultats à ceux des inventaires acoustiques de densité de biomasse du krill réalisés durant les mêmes années dans la région occidentale de la mer de la Scotia par le programme américain « Antarctic Marine Living Resources » (AMLR). Les densités de biomasse du krill près de la côte sont significativement supérieures (test de t, $p < 0,05$), plus stables et leurs coefficients de variation plus petits que dans les inventaires beaucoup plus étendus de l'AMLR. La compétition accrue entre les phoques, les manchots et les humains pour la ressource de krill côtier, surtout durant les mois de l'été austral, pourrait affecter le succès du recrutement de ces prédateurs de krill venus du continent. Nous discutons des implications de la biomasse du krill côtier sur les unités de gestion à petite échelle.

[Traduit par la Rédaction]

Introduction

Antarctic krill (*Euphausia superba*) are the main food resource for many of the fish, seabirds, and marine mammals in the Southern Ocean ecosystem. In addition to its ecological importance (Marr 1962; Croxall et al. 1999; Siegel 2000), krill are also the basis of a commercial fishery (Agnew 1997; Jones and Ramm 2004). To effectively manage this fishery, accurate estimates of the abundance and distribution of Antarctic krill are required. Towards this goal, several nations, as part of the Commission for Conservation of Antarctic Marine Living Resources (CCAMLR), conduct surveys of the krill population using both echosounders and net tows to measure the krill population (Hewitt and Demer 2000). The results of these surveys are then used to set precautionary catch limits for the fishers (Hewitt et al. 2004*b*).

Received 15 June 2009. Accepted 5 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 24 June 2010.
J21254

Paper handled by Associate Editor J. Michael Jech.

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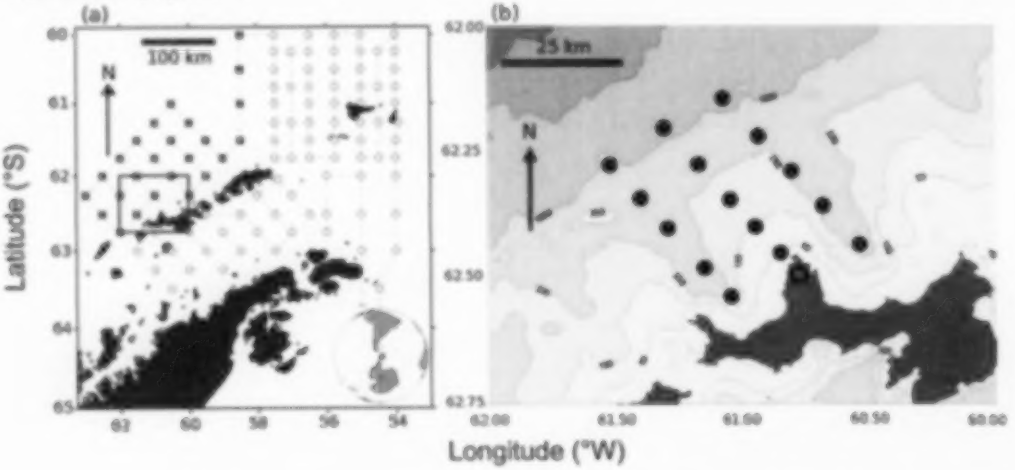
Can. J. Fish. Aquat. Sci. **67**: 1159–1170 (2010) doi:10.1139/F10-042 Published by NRC Research Press

Fig. 1. (*a*) The US Antarctic Marine Living Resources (AMLR) program conducts an annual survey of krill abundance with conductivity–temperature–depth (CTD) and net tow stations (squares and circles) and multiple-frequency acoustic transects between stations. The western area of the AMLR survey is indicated by shaded squares. (*b*) An expansion of the box in (*a*) delineating the nearshore survey area. The locations of CTD and net tow stations of the nearshore survey are indicated by solid circles. The Cape Shirreff field camp (square) was the port for the nearshore survey operations.



In the Scotia Sea region of the Southern Ocean (UN Food and Agriculture Organization's statistical area 48), the US Antarctic Marine Living Resources (AMLR) program has conducted annual krill surveys for more than two decades (Hewitt and Demer 1991; Hewitt et al. 2003). To survey this very large area (Fig. 1), a series of transect lines and more than 100 sampling stations are conducted at least once per year during the austral summer (Hewitt et al. 2003).

The AMLR survey data from 1999 and 2000 were combined with the results of surveys from multiple other nations and identified three areas of consistently high krill biomass in the vicinity of the South Shetland Islands: eastern end of Elephant Island; midway between Elephant Island and King George Island; and the north side of Livingston Island (Hewitt et al. 2004*a*). However, the large-area surveys did not sample much of the shallow, on-shelf regions of the South Shetland Islands or Antarctic Peninsula. Because the nearshore areas have a much smaller spatial area (relative to the offshore large-area surveys), it is unlikely that higher nearshore krill abundances would affect annual estimates of krill biomass. However, the importance of the nearshore areas to predator foraging, fishing, and thus small-scale management units (CCAMLR 2006) may be disproportionately large.

Nearshore regions are defined here as areas on the continental shelf that are within tens of kilometres of land. These regions are important areas for several land-based species (e.g., penguins (*Pygoscelis* spp.) and Antarctic fur seals (*Arctocephalus gazella*)) that have established colonies and rookeries in the South Shetland Islands and, in particular, Livingston Island (Boveng et al. 1998; Croll and Tershy 1998). During the austral summer months, the adult animals regularly forage in and transit through the nearshore area of Cape Shirreff to provide food for their young on land.

The abundance and behavior of several different types of krill predators (e.g., flying sea birds, penguins, and fur seals) in the nearshore area are affected by changes in nearshore krill biomass (Miller and Trivelpiece 2008; Warren et al. 2009). However, the relationship between the breeding success of these land-breeding predators and the offshore krill abundance may be complex. For example, Croll et al. (2006) found that penguin reproductive success was correlated with offshore krill abundance, but penguin foraging effort was not. These findings suggest a possible lack of coherence between the nearshore and offshore krill abundances. If nearshore krill biomass is sufficiently large and annually stable, this could explain the long-term persistence of penguin and seal breeding colonies along the Antarctic Peninsula (Croll and Tershy 1998; Hinke et al. 2007).

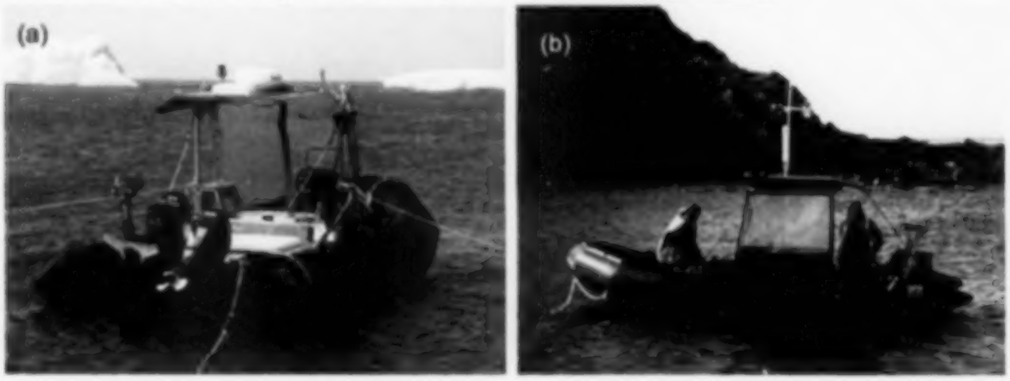
Alternatively, if krill abundance within the foraging ranges and during the breeding season of penguins and seals is reduced by natural variability, climate change, or fishing effort, the apparent ecosystem balance could falter. Much of the commercial krill fishing takes place in these nearshore areas of the Antarctic Peninsula (Jones and Ramm 2004) and may directly compete with foraging seals and penguins, particularly during their breeding season. As both the native animals (fish, seabirds, and marine mammals) and humans are consuming the limited krill resource in the nearshore areas, increased competition may negatively impact the reproductive success of animals confined to forage from their land breeding sites.

To explore the hypothesis that nearshore krill biomass density is larger and more stable interannually than the offshore krill biomass density, a method was developed and a series of surveys were conducted to measure the abundance and distribution of krill in the nearshore region north of Cape Shirreff, Livingston Island, Antarctica. Cape Shirreff is the site of several colonies of Antarctic fur seals and chinstrap (*Pygoscelis antarctica*) and gentoo (*Pygoscelis papua*) penguins. The AMLR field camp there served as a base for the nearshore survey operations. Because of the shallow depths and lack of accurate bathymetric charts of the area,

Table 1. Nearshore survey timing and duration, total length of all transects, depths sampled by acoustic echosounder, coverage of canyons east and west of Cape Shirreff, and timing of echosounder calibration.

Date	Survey length (km)	Depth recorded (m)	Coverage	Echosounder calibration
6–10 February 2000	148	250	West, east	Before
17–23 February 2002	250	100	West, east	Before
7–09 February 2004	130	250	East	During
1–10 February 2005	280	400	East	Before and after
3–08 February 2006	234	400	East	Before and after
29–30 January 2007	77	400	East	Before

Fig. 2. (*a*) R/V *Ernest I* was used to survey the nearshore waters of Livingston Island for krill abundance and distribution in 2000, 2002, and 2004. It contained a single-frequency (120 kHz) split-beam echosounder, a meteorological station (not shown), and an electric downrigger for cast deployments of a CTD. (*b*) R/V *Ernest II* was used in 2005–2007 and contained a multiple-frequency (38 and 200 kHz) single-beam echosounder, a sea-surface temperature and salinity sensor, and a meteorological station.



instrumented small craft were developed and used to study the previously inaccessible nearshore region.

Materials and methods

During six austral summers (late January and early February) of 2000, 2002, and 2004–2007, an instrumented small craft (R/V *Ernest*) was used to survey the area surrounding Cape Shirreff (Table 1). This area is characterized by shallow (less than 100 m) depths except for two large submarine canyons that exist to the northeast and northwest of the Cape (Fig. 1). These canyons extend to the shelf break approximately 30–40 km offshore. The bathymetry of the non-canyon regions contains many pinnacles and other pronounced underwater features, some extending to within a few metres of the surface. There is also a small sheltered cove with a rocky beach that served as an anchorage for R/V *Ernest*. Transit to and from Cape Shirreff was on R/V *Yuzhmorgeologiya* during the first leg of the annual AMLR survey of the Scotia Sea krill population. The duration of each nearshore survey ranged from 2 to 10 days (Table 1), depending on several factors including the duration of the AMLR cruise, weather delays during the large-area survey, and weather and sea conditions during the transfer to and from the field site and during the nearshore survey period.

R/V *Ernest*

Two people (captain, scientist) served as crew for R/V *Ernest*, which had two distinct forms during this study (Fig. 2). During the first three field seasons, an aluminum pilothouse was fitted into the 6 m inflatable (Zodiac Mark V) boat. The insert contained batteries supplying power to a variety of communication and safety equipment (radar, VHF radio, radar, EPIRB), as well as scientific instruments. Sensors onboard the vessel included a meteorological station (WeatherPak 2000; Coastal Environmental Systems Inc., Seattle, Washington), which measured temperature, humidity, barometric pressure, bearing, and apparent and true wind speed and direction; multiple global positioning system (GPS) receivers; and a 120 kHz split-beam echosounder (Simrad EY500; Kongsberg Maritime AS, Horten, Norway). GPS, meteorological, and acoustic backscatter data were recorded on a laptop computer. A liquid-crystal display, mounted behind a waterproof window, provided the scientist with a real-time display of position and meteorological and echosounder data. A motorized downrigger was used to deploy a small conductivity–temperature–depth (CTD) profiler (SBE 19; Sea-Bird Electronics, Inc., Bellevue, Washington) and an underwater video-camera system (Sony Handycam with Light and Motion Stingray housing and lights; Sony Inc., Tokyo, Japan) at stations throughout the survey area. The video camera was used to confirm the classifications of acoustically detected targets. The boat was also equipped with survival and tool kits, manual and automatic bilge pumps, three survival suits, four fuel tanks, binoculars, and anchorage equipment. Transit operations used a 55-hp gas outboard engine, whereas survey operations used a 9.9-hp outboard engine with generator to allow remote steering, conserve fuel, charge batteries, and minimize noise in the acoustic record. During the 2000 and 2002 field seasons, the transducer was deployed on a portside gimbal mount; however, transducer movement in certain sea conditions reduced data quality so a transom-mounted transducer arm was installed in 2004 and used thereafter.

For the 2005–2007 field seasons, the aluminum insert was replaced by two waterproof equipment cases and a dodger made of stainless steel, canvas, and vinyl (Fig. 2). These changes reduced the weight of the boat, allowed the vessel to plane when transiting between survey locations, and provided more protection from the elements for the crew. The smaller engine was removed and the larger one was used for both transiting and survey operations. This change allowed for faster survey speeds and an increase in survey coverage. The single-frequency echosounder was replaced with 38 kHz and 200 kHz echosounders (SIMRAD dual-frequency single-beam ES60; Kongsberg Maritime AS), which used a 802.11 g wireless network to link the echosounder to a laptop computer on *Ernest*. In addition, a temperature and salinity sensor (SBE 37 microCAT; Sea-Bird Electronics, Inc.) was deployed on the transom-mounted transducer arm to record subsurface (1 m) hydrography during the survey.

Survey operations

Meteorological and position data were recorded whenever the boat was transiting or surveying. The vessel transited at high speeds (ca. 8 $m \cdot s^{-1}$) to the beginning of a transect line. The transducer arm was then lowered and acoustic and hydrographic data recording commenced. Depending on the track lines to be run each day, transits between track lines were made either slowly (2–5 $m \cdot s^{-1}$) while recording echosounder data or rapidly with the transducer raised out of the water. Meteorological, date, time, and position data were recorded every 20 s on the laptop computer. Hydrographic data (temperature, salinity, and pressure) from the CTD were recorded every 5 s. Occasionally, when large aggregations of scatterers were observed on the echosounder near the surface (<75 m depth), the digital video camera was lowered to record images and identify the scatterers. Weather conditions, sea state, fuel supply, and crew endurance determined the length of survey operations each day, but typically 5 to 8 h each day were spent collecting data and transiting. Surveys were conducted in all types of weather (often in the same day) and were suspended when seas grew greater than 4 m, visibility was reduced to less than 10 m, or winds exceeded those for safe vessel operation (e.g., >10 $m \cdot s^{-1}$).

The survey transects varied in length and direction during the first few years of this study, as the operational capabilities and limits of *Ernest* were identified. Also, as measurements from *Ernest* were used to map the nearshore bathymetry, increasing portions of the nearshore area were safely surveyed from *Yuzhmorgeologiya*. The survey strategy soon stabilized, and since 2004, nearly identical transect legs east of Cape Shirreff were surveyed from *Ernest*. Transits to the western side of Cape Shirreff can be difficult in certain sea-state or fog conditions, so efforts were focused on a higher spatial coverage of the eastern side of the Cape when survey time became limited during the 2004 field season. The direction of the transects during the 2004 to 2007 surveys were chosen to align with cruise tracks that *Yuzhmorgeologiya* surveyed during the large-area survey so that comparisons could be made between the data collected from the two observational platforms. However, survey tracks (direction and distance) were often adjusted depending on the direction and size of the waves and wind. The 2004 nearshore survey consisted of only 3 days of operations due to severe weather and sea conditions during 4 days of the scheduled weeklong survey period. Similarly, the 2007 nearshore survey was reduced to less than 2 days as a result of severe weather during both the large-area and nearshore survey periods.

Acoustic data

Calibration

The echosounders were calibrated for each survey using the standard sphere method (Foote et al. 1987) with a 38.1 mm tungsten carbide sphere (Table 1). Using a single monofilament tether, the sphere was lowered beneath *Ernest* until it appeared on the echogram and the target strength (TS), uncompensated for beam directivity effects (TS_U; dB), was maximized. Several hundred TS_U measurements of the sphere were recorded at this depth while both the sphere and transducer moved with water and vessel motion. For the 120 kHz split-beam measurements, the sphere position in the beam was measured and the TS_U were compensated for the beam directivity (dB). In the cases of the single-beam 38 kHz and 200 kHz transducers, the location of the sphere within the acoustic beam was unknown. For most of the single-beam calibration experiments, the distributions of TS measurements were examined to confirm that some on-axis measurements were achieved. These measurements were used to calculate the on-axis calibrated system gain (Foote et al. 1987).

In 2005, on-axis calibration measurements were obtained for the target for both frequencies of the echosounder. However, in 2006, because of strong currents, TS measurements were intermittent with the narrower beamwidth (7°) of the 200 kHz transducer, but continuous with the wider beamwidth (13° longitudinal and 21° transverse) of the 38 kHz transducer. In this case, theoretical beampatterns were calculated for both transducers, and the differences in TS_U at the two frequencies were predicted for a range of off-axis angles. This information was used to constrain the possible locations for the sphere within the beam. The data were then fit to the theoretical predictions using the method of least squares to estimate the minimum off-axis angle achieved (i.e., 5°). The TS measurements were then compensated for the beampattern and used to estimate the calibrated echosounder gain. In 2007, a calibration was not possible due to the short survey duration and inclement weather and sea-state conditions. Consequently, the 2006 settings were used for the 2007 survey. For the first four field seasons, the calibration accuracy is estimated to be better than 1 dB (one-way gain accuracy $\leq$ 0.5 dB or ~12%). For the 2006–2007 field seasons, the aforementioned procedure is thought to result in calibration accuracy of better than 2 dB for each frequency (one-way gain accuracy $\leq$ 1.0 dB or ~26%).

Data collection

Survey speeds varied depending on sea state, wind, and

Table 2. Conversion of acoustic backscatter data to estimates of krill biomass density used krill length information from net tows and the SDWBA scattering model to determine the criteria to identify backscatter from krill.

		Target strength (dB)				
		Smallest krill		Largest krill		
Year	Range of krill lengths (mm)	38 kHz	200 kHz	38 kHz	200 kHz	ΔS_v range used to identify krill
2005	40–55	–81.3	–74.4	–73.0	–71.2	2.6–7.0
2006	45–58	–78.7	–73.5	–73.6	–71.5	2.0–5.2
2007	22–60	–94.5	–79.3	–74.5	–71.9	1.8–15.2

Note: ΔS_v is the S_v at 200 kHz minus the S_v at 38 kHz. This method was only applied to the data from 2005 to 2007 as the 2000, 2002, and 2004 surveys used a single-frequency echosounder.

transducer-mount location but were between 2 and 4 m·s^{-1}. The transducer was located between 1 and 1.75 m beneath the surface. Acoustic pulses of 1.024 ms duration were transmitted every 1 s at 120 kHz (2000, 2002, and 2004) and 2 s at 38 and 200 kHz (2005–2007), and volume backscattering strengths (S_v; dB re 1 m^{-1}) were recorded every 20 to 30 cm from the transducer to depths of 250 m (2000), 100 m (2002), 250 m (2004), and 400 m (2005, 2006, and 2007) (Table 1). All locations in the survey area were shallower than 400 m, and only the middles of the canyons were deeper than 250 m.

Acoustic backscatter provides indirect measures of the distribution and abundance of biological organisms in the marine environment. Validation of this metric was achieved with a series of 2.5 m^2 Isaac–Kidd midwater trawl net tows during the 2002, 2004, and 2005–2007 field seasons. The tows were conducted from *Yuzhmorgeologiya* in adjacent areas (Fig. 1). The net-tow contents were enumerated and identified to species onboard *Yuzhmorgeologiya* and then preserved in a buffered (10%) formalin solution. During the 2005–2007 surveys, adult Antarctic krill were sexed and their lengths were measured before preservation. At least once during each field season (except for 2004 and 2007), an underwater video camera was lowered into a patch of near-surface scatterers that were observed on the echosounder. *Ernest* drifted as the camera was lowered to the depth of the scattering aggregation and then retrieved. This method was occasionally unsuccessful if the scatterers dispersed or the aggregation moved while the camera was lowered. Successful video observations indicated that aggregations of large euphausiids (likely *E. superba*) were the dominant mesozooplankton in the water column.

Volume backscattering coefficients (s_v (m^{-1}), where S_v = 10·log$_{10}$(s_v)) were summed from 3 to 10 m below the sea surface to the shallower of 100 m or 1 m above the seafloor (observation range common to all years) and averaged over 185 m (0.1 nautical miles (n.mi.)) trackline distances, yielding nautical area backscattering coefficients (s_A; m^2·n.mi.$^{-2}$). Selected regions were excluded from the integration, including the acoustic near-field and scatter from nonbiological sources such as bubbles, suspended sediments (e.g., mud or sand), or the seafloor (particularly the dead zone above high-relief hard substrates; see Demer et al. 2009). In the 2004–2007 data, noise from the engine was also removed by coherent subtraction (Hewitt et al. 2004*b*) and filtered by thresholding below S_v = –80 dB re 1 m^{-1}.

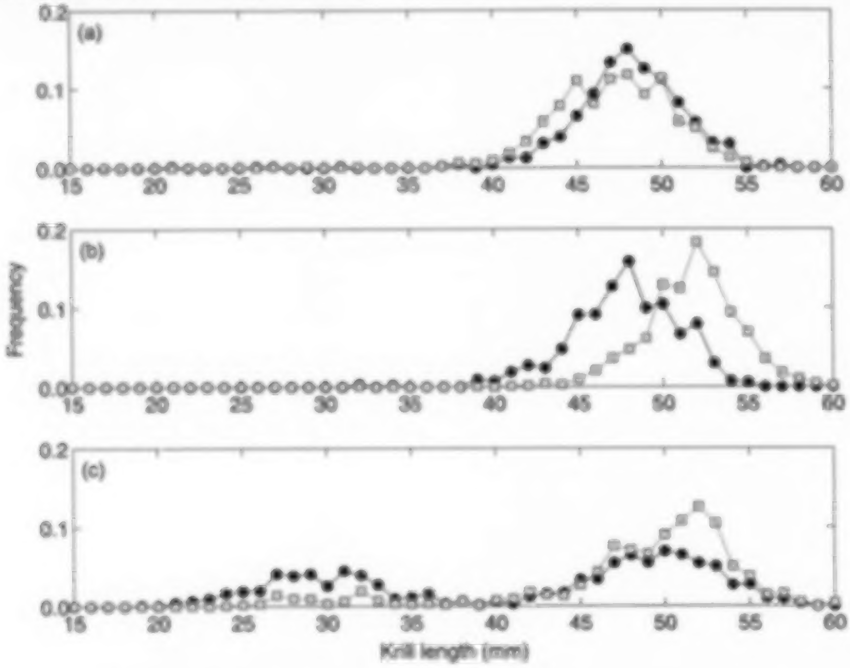
Conversion of backscatter data

The dual-frequency echosounder data were filtered using the ΔS_v method (Watkins and Brierley 2002; CCAMLR 2005) prior to the calculations of s_A. The ΔS_v method aims to accept S_v from krill and reject that from other biological scatterers. Following the procedure of CCAMLR (2005, 2009), the minimum and maximum length of krill caught in the net samples for each year were used to calculate the ΔS_v range for attributing acoustic backscatter to krill. The stochastic distorted wave Born approximation (SDWBA) model (Demer and Conti 2005; Conti and Demer 2006; CCAMLR 2009) was used to calculate the predicted difference in scattering (TS at 200 kHz minus TS at 38 kHz) for the smallest and largest krill lengths. These two ΔS_v values formed the upper and lower bounds for filtering the acoustic backscatter data (Table 2). This technique could not be applied to the single-frequency 120 kHz data collected during the 2000, 2002, and 2004 nearshore surveys.

The s_A were converted to estimates of krill biomass density using the length distribution of krill caught in nets and a theoretical acoustic scattering model (Demer and Conti 2005; Conti and Demer 2006; Reiss et al. 2008) that has been adopted by CCAMLR as the standard method for producing estimates of krill biomass (CCAMLR 2005, 2009). Because the nearshore trackline lengths and directions were not standardized for all survey years, the method of Jolly and Hampton (1990) was used to measure the mean weighted density and coefficient of variation (CV) for a series of transects comprising the survey for each year. These values were then comparable between the six nearshore surveys and the AMLR Scotia Sea surveys, which were analyzed in a similar manner (method 3 from Reiss et al. 2008).

Comparisons of the nearshore survey results between years are complicated by the different acoustic instrumentation (dual versus single frequency), differing observational depths, and different amounts and locations of survey effort. To quantify the potential bias in the 120 kHz data (2000, 2002, 2004) due to the possible inclusion of non-krill scatterers, the 200 kHz data (2005–2007) were processed with and without the ΔS_v method and compared. The s_A at 120 kHz were then corrected using the relationship found using the 200 kHz data. Although there can be significant differences between the TS at 120 and 200 kHz for a single animal (Demer and Conti 2005), krill aggregations exhibit a range of lengths (as measured from animals caught in net tows) and orientations relative to the incident sound wave.

Fig. 3. Length distributions of *Euphausia superba* collected from net tows from (*a*) 2005, (*b*) 2006, and (*c*) 2007 during the nearshore survey (shaded squares) and AMLR western area (solid circles).



Thus the mean TS for a krill aggregation can be calculated by weighting the orientation-averaged backscattering cross section for krill at each length by the proportion of krill of this length caught in the net. This has the effect of dampening the TS versus length oscillations in the geometric scattering regime (where the krill length is large relative to the acoustic wavelength).

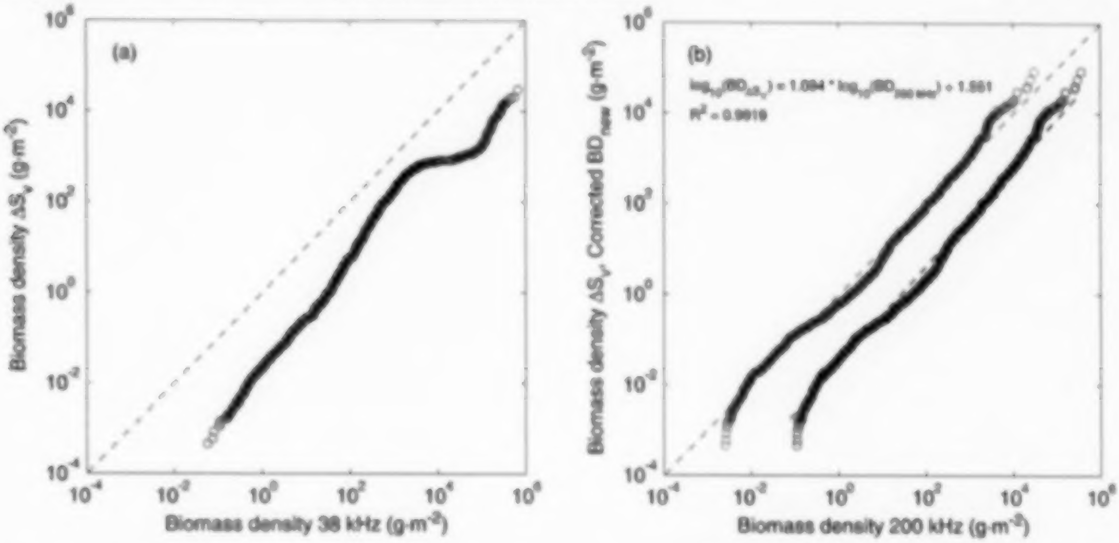
Because S_v data were recorded to different maximum depths (Table 1), the minimum requisite survey depth was explored. Most of the scattering was observed in the upper 100 m of the water column, so the effect of not integrating S_v deeper than 100 m is assumed to be insignificant. This assumption is supported by independent observations of krill throughout the Scotia Sea during the austral summer of 2000 (see fig. 9 in Demer 2004). To further validate this assumption, the scatterer aggregations in the 2005 and 2006 data that were identified as krill using the ΔS_v method were further characterized with regard to their size and depth.

Results

Net-tow samples for all years indicate that adult and juvenile *Euphausia superba* were the largest contributors to zooplankton biomass in this region, although other smaller euphausiids (*Thysanoessa macrura*, *Euphausia frigida*) were also frequently caught in smaller numbers. Other zooplankton and nekton in the net catches were copepods (*Metridia gerlachei*, *Calanoides acutus*, *Calanus propinquus*, *Rhincalanus gigas*, and *Paraeuchaeta* spp.), salps (*Salpa thompsoni*), amphipods (*Cyllopus lucasii*, *Primno macropa*, and *Themisto gaudichaudii*), chaetognaths, larval fish (*Lepidonotothen larseni* and *Electrona* spp.), and gastropods (*Limacina helicina*, *Spongiobranchaea australis*, and *Clione limacina*). The three taxa that accounted for the overwhelming majority of biomass were Antarctic krill, copepods, and salps. Neither copepods nor salps are considered to be strong acoustic targets at these frequencies compared with the krill (Demer 1994; Stanton et al. 1996; Stanton and Chu 2000), so these acoustic estimates of biomass should not be strongly affected by the presence of either. Larval fish, however, are relatively strong scatterers, but they were found infrequently in the net tows, generally only at nighttime, and always in low numerical densities. Pteropods are also strong scatterers; however, they were only found in the shelf-break net tows (i.e., those farthest from the nearshore survey region) and were caught in very low numerical abundances, so their contributions to the nearshore backscatter are likely to be small.

A key factor in the conversion of s_A to estimates of biomass is the estimated probability density function (pdf) of krill lengths. This length pdf is used to calculate both the weighted scattering cross-section and the biomass per krill. For 2000, 2002, and 2004, the length pdfs for the nearshore area were assumed equivalent to those measured in the western area of the AMLR broad-scale survey, which includes the South Shetland Islands. For the 2005–2007 surveys, the length pdfs were calculated from net samples from the nearshore area (Fig. 3). In 2005, the nearshore and AMLR western area distributions were nearly identical (coefficient of determination, $R^2 = 0.96$), whereas the 2006 ($R^2 = 0.52$) and 2007 ($R^2 = 0.81$) distributions showed slightly (approximately 5 mm) larger krill in the nearshore waters. Additionally, in 2007, smaller (juvenile) krill were caught in both regions, although the nearshore region had fewer of these

Fig. 4. Comparison between values of integrated krill biomass density (BD) (g·m^{-2}) (circles) estimated from single-frequency backscatter data at (*a*) 38 kHz and (*b*) 200 kHz ($BD_{38 kHz}$ and $BD_{200 kHz}$, respectively) and from the two-frequency ΔS_v method ($BD_{\Delta S_v}$). The value of the single-frequency estimates is on the horizontal axis, while the two-frequency ΔS_v estimates are on the vertical axis. The shaded broken line in both plots represents where the single-frequency and ΔS_v method estimates of biomass density are equal. To correct single-frequency survey data (which cannot discriminate between krill and non-krill scatterers), an equation (shown in (*b*)) was found that related $BD_{200 kHz}$ and $BD_{\Delta S_v}$, which was used to produce corrected 200 kHz biomass estimates (BD_{new}, shaded circles). The black broken line in (*b*) is a linear regression fit to the data that was used to calculate the correction function.



younger krill. Given the general agreement between the nearshore and western area distributions in the 2005–2007 surveys, errors resulting from using the western area length pdfs in 2000, 2002, and 2004 are likely small.

Using the 38 kHz and 200 kHz data from 2006, biomass densities calculated from the 38 or 200 kHz data, without the ΔS_v method, were, as expected, higher than those derived with the dual-frequency algorithm (Fig. 4). Therefore, the biomass densities estimated from the single-frequency data collected in 2000, 2002, and 2004 are probably overestimated. To account for this bias, a correction factor was developed and applied. First, biomass densities were calculated for each of the 2005–2007 surveys using only the 200 kHz data (i.e., replicating a single-frequency echosounder) and using both frequencies (i.e., the ΔS_v method). The differences between the biomass densities calculated using the single-frequency (200 kHz) and ΔS_v methods were fairly uniform across all values of measured backscatter (Fig. 4*b*) and for each survey. The 2006 data included the largest range of biomass density values and were therefore used to estimate the correction factor, but the 2005 and 2007 data had the same pattern where they overlapped. This suggests that the ΔS_v method, tuned by the krill length pdfs, is not affected by the overall level of backscatter and that scatter from small (e.g., salps and smaller euphausiids) and large organisms (fish) was rejected, as desired, using the ΔS_v method.

The length-weighted TS for krill at 120 kHz for each year of the survey using the SDWBA model (Conti and Demer 2006) were 0.45 ± 0.15 dB larger than that at 200 kHz. To account for this small, expected difference, the S_v at 120 kHz were reduced by 0.45 dB before calculating the s_A (Demer 2004). Next, a correction formula was found for the 2006 data by fitting a straight line (in log–log space) to the 200 kHz biomass density data and then transforming these data to match the biomass density using the ΔS_v method (Fig. 4). The correction equation, based on the 200 kHz biomass density data, was applied to the adjusted 120 kHz biomass density ($BD_{120 kHz}$) from 2000, 2002, and 2004 data, resulting in a revised biomass density estimate ($BD_{120 new}$) that was used in all subsequent analyses (Fig. 4): $BD_{120 new} = 10^{-1.551} \cdot BD_{120 kHz}{}^{1.084}$.

During each nearshore survey, krill biomass density was observed to be high throughout the shallow regions proximate to Cape Shirreff (Fig. 5). High biomass densities were mapped both along the edges and within the canyon east of Cape Shirreff. Areas to the southeast and south of the eastern canyon had relatively lower biomass. Coupling the 2000 and 2002 *Ernest* surveys of the canyon west of Cape Shirreff with observations from *Yuzhmorgeologiya* (Warren et al. 2009), there is an indication that krill are equally as abundant there as in the eastern canyon.

In addition to examining horizontal distributions, the vertical distributions of krill aggregations were examined in both 2005 and 2006. Unlike the echo-integration analyses, which were limited to the upper 100 m of the water column, krill aggregations were examined throughout the water column (as the maximum observational depth of 400 m exceeded the maximum bottom depth in these surveys). Aggregations of krill were identified from acoustic-backscatter data within the ΔS_v range corresponding to the range of krill lengths observed each year. Data from both years showed that krill aggregations had similar depth distributions with mean (±1 standard deviation, SD) depths of 62 (±26) m in 2005 and

Fig. 5. Spatial distributions of krill biomass density (g·m^{-2}) estimated from acoustic backscatter sampled during (*a*) 2000, (*b*) 2002, (*c*) 2004, (*d*) 2005, (*e*) 2006, and (*f*) 2007 of the Livingston Island nearshore survey. The 200 m isobath (thin black line) marks the edge of the submarine canyon where large krill aggregations were regularly found.

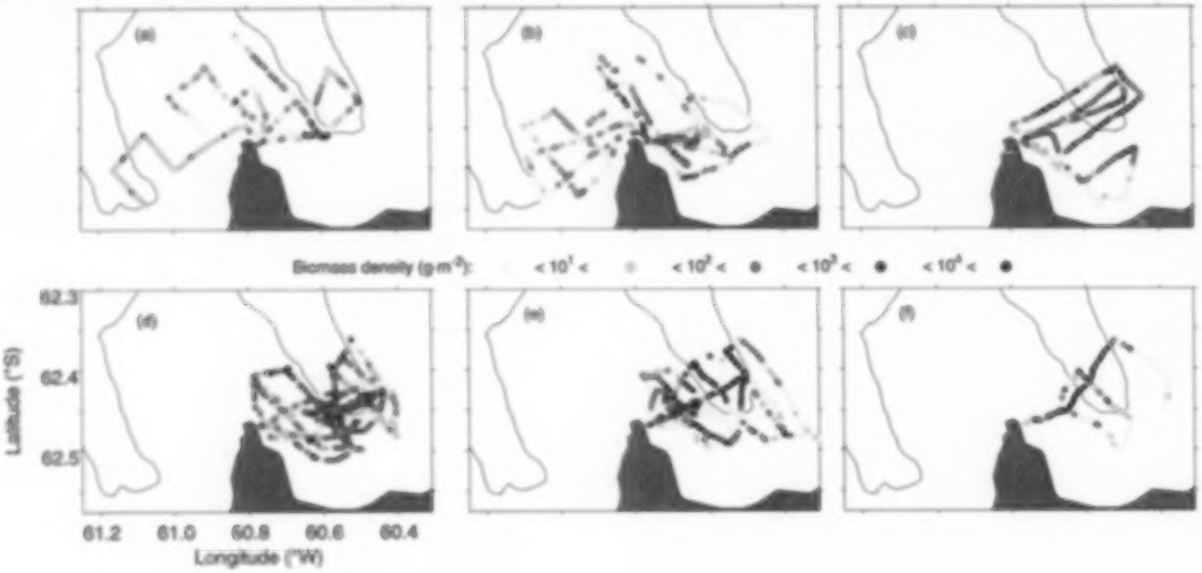
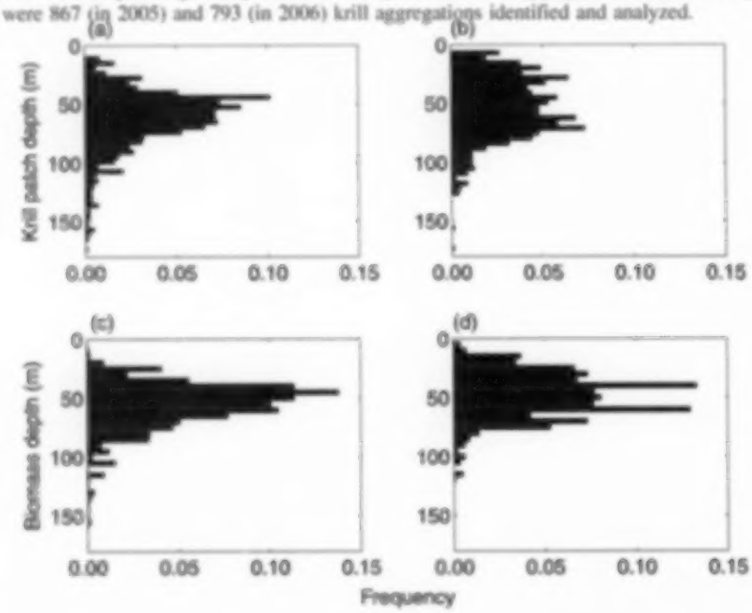


Fig. 6. Distributions of the depth of the center of individual krill aggregations identified during the (*a*) 2005 and (*b*) 2006 nearshore surveys and the depth of the center of mass (depth weighted by biomass density) of each krill aggregation sampled during the (*c*) 2005 and (*d*) 2006 nearshore surveys. There were 867 (in 2005) and 793 (in 2006) krill aggregations identified and analyzed.



53 (±27) m in 2006. The center of mass of krill aggregations (e.g., depth of krill aggregations weighted by the biomass of the swarm) was 55 m in 2005 and 48 m in 2006. During both 2005 and 2006, the overwhelming majority of krill aggregations (92% and 95%, respectively) and their biomasses (97% and 99%, respectively) were located at depths less than or equal to 100 m (Fig. 6).

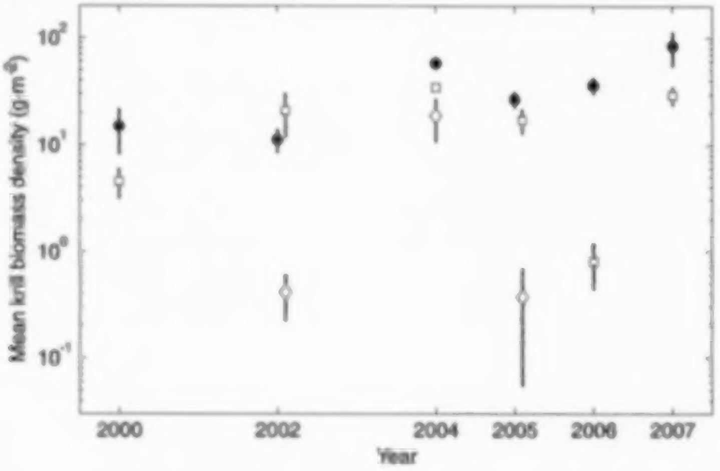
To compare estimates of biomass from each survey, a series of roughly parallel transects were selected from each nearshore survey. The transect directions were not the same each year because of the different survey designs; however, for the last three years of the survey, the transects were roughly oriented northwest to southeast. Following Jolly and Hampton (1990), the mean biomass density for each

Table 3. Comparison of mean krill biomass densities (BD) and coefficient of variation (CV) for the transects from the nearshore survey and the AMLR Western Area (AMLR 2008), and the number of nearshore transects and total transect distances used to calculate BD.

	Nearshore survey				AMLR Western area	
Year	Transects (number)	Distance (km)	BD ($g \cdot m^{-2}$)	CV (%)	BD ($g \cdot m^{-2}$)	CV (%)
2000	9	93	14.9	46	4.51 (D)	32.2 (D)
2002	11	103	11.2	25.6	21.02 (A)	44.6 (A)
					0.41 (D)	46.4 (D)
2004	7	84	57.9	13.2	34.37 (A)	8.9 (A)
					18.87 (D)	44.0 (D)
2005	24	222	26.9	17.1	17.11 (A)	26.6 (A)
					0.37 (D)	85.2 (D)
2006	17	162	36.2	17.5	0.81 (A)	45.9 (A)
2007	3	32	84.3	36.1	29.23 (A)	19.7 (A)

Note: Analyses of both the AMLR Western Area and nearshore data use the same SDWBA scattering model and procedure for converting acoustic backscatter to krill biomass. AMLR surveys occurred once or more each year in January (A) or February (D). Biomass densities from 2000 to 2004 (single-frequency data) were corrected using the relationship shown in Fig. 4.

Fig. 7. Annual mean krill biomass density ($g \cdot m^{-2}$) and coefficient of variation (whiskers) for the nearshore survey (solid circles) and the western area of the AMLR Scotia Sea January (open squares) and February (open diamonds) surveys. For points where the uncertainty is not visible, the CV is smaller than the marker.



segment and the transect lengths were calculated. The mean biomass density for each transect, weighted by transect length, were then calculated for each survey along with their coefficients of variation (CV values; Table 3; Fig. 7). The CV values were generally similar between years, except for 2000 and 2007, which were higher. In 2000, this is likely the result of the longest transect in that survey having a mean biomass density six times larger than the other transects. In 2007, the large CV is due to the short duration of the survey and, consequently, only three transects available for analysis.

Annual mean krill biomass densities ranged from 11 to 84 $g \cdot m^{-2}$ and were significantly larger than krill biomass density estimates from the AMLR western area survey (*t* test, $p < 0.05$; Table 3; Fig. 7). The estimates of biomass density in 2004 and 2007 (57.9 and 84.3 $g \cdot m^{-2}$, respectively) were substantially larger than those for the other years, motivating a thorough re-examination of the echograms. The high biomass estimates were the result of krill aggregations in both years being both more numerous and dense. In every year except 2002, the nearshore biomass density was greater in the nearshore region than in the western area of the AMLR survey (AMLR 2008), and in some years, nearshore biomass densities were substantially greater than those offshore (Table 3; Fig. 7). The mean nearshore biomass density estimates were larger (mean, 38.6 $g \cdot m^{-2}$; SD, 28.0 $g \cdot m^{-2}$) and always greater than 11 $g \cdot m^{-2}$, whereas the western area estimates were smaller (mean, 14.1 $g \cdot m^{-2}$; SD, 13.1 $g \cdot m^{-2}$), exhibited a much larger variance from year to year, and in some years were very small (i.e., less than 1 $g \cdot m^{-2}$). Based on these six years of survey data, krill biomass densities remained high in the nearshore regions even when the offshore krill biomass densities were low.

Discussion

This multiyear study has demonstrated that a small boat equipped with a scientific echosounder and either video or nets to verify the acoustic scatterers as krill can successfully survey shallow, nearshore waters of Antarctica. The estimates of nearshore krill biomass density are significantly larger, exhibit smaller interannual changes, and have smaller CV values within each survey year than the acoustic surveys of the much larger offshore areas of the Scotia Sea. For the area north of Cape Shirreff, during the austral summers of the years of this study, krill were consistently found in similar areas, had a minimum biomass density of at least 11 $g \cdot m^{-2}$, and had mean biomass densities that were much larger than the adjacent offshore waters in all but one year. The western area of the AMLR survey often had biomass densities that were one order of magnitude smaller than the nearshore areas, although in some years, biomass differences were even greater. Physical (advection) and biological (upwelling, increased phytoplankton biomass) factors in the nearshore area are possible reasons for the consistently higher krill biomass in this region.

Note that the AMLR survey data collected at three acoustic frequencies (38, 120, and 200 kHz) are currently analyzed using two ΔS_v ranges to identify acoustic scattering from krill (e.g., Reiss et al. 2008), following CCAMLR (2005, 2009). These differences in survey data and analysis techniques may partially explain their smaller estimates of krill biomass density. However, because of the absence of small krill in the nearshore net tows, the nearshore survey had a much smaller ΔS_v range (except in 2007 when the ranges are comparable) than that used by the AMLR survey. Consequently, the data presented here are conservative estimates of the nearshore krill biomass density (CCAMLR 2009). In addition to the nearshore waters having larger and more uniform krill densities compared with the nearby offshore region, the nearshore waters generally contain more large adult krill and fewer juvenile krill. Thus, the nearshore region may avail the land-based predators with higher-quality prey (i.e., more biomass per krill). Why larger krill preferentially aggregate nearshore is yet to be discovered.

Although the sampling methods and instrumentation changed somewhat between the nearshore surveys, the differences probably do not affect the overall results. The mean krill biomass densities from 2000, 2002, and 2004 are similar (and in general smaller) than those from the years when the dual-frequency discrimination was done, suggesting that our method of correcting these data did not produce positively biased results. The different integration depths used for different years do not contribute appreciable biases because the majority of the krill biomass was in the upper 100 m of the water column and all the integration depths were greater than or equal to 100 m. These distributions are similar to that observed for krill surveyed over the entire Scotia Sea during 2000 (Demer 2004) and in other studies of Antarctic krill (Miller et al. 1993; Siegel and Kalinowski 1994). Moreover, Miller and Trivelpiece (2008) found that chinstrap penguins did not exhibit foraging dives deeper than 93 m (2005) and 88 m (2006) during the day, which suggests that sufficient krill prey resources exist shallower than 100 m.

The relative importance of nearshore krill in terms of the total krill stock in the Scotia Sea (e.g., Hewitt et al. 2004*b*) is unknown; however, the spatial area of this nearshore survey is quite small (~200 km^2) when compared with that of the Scotia Sea. The relatively high and stable densities of krill biomass in the nearshore regions of the South Shetland Islands may be important to include in some estimates of krill biomass, particularly in subregions considered for small-scale management units. Although the three identified "hot spots" of krill biomass from the CCAMLR 2000 multinational, Scotia Sea survey (Hewitt et al. 2004*a*; CCAMLR 2006) are all proximate to nearshore areas, the surveys used to identify these hot spots did not extensively sample the shallower nearshore waters, as was done in this study. The large nearshore krill biomass is generally most accessible and attractive to the land-breeding predators, as well as to human fishers competing for this valuable resource. Animals and fishers alike are drawn to the nearshore hot spots during the austral summer months (Jones and Ramm 2004). Given the different objectives of research vessels (standardized survey effort to collect high quality data) and fishing vessels (maximize profits by catching more animals), fishing vessels may be willing to work closer to land than research vessels, which may increase competition between land-based predators and fishers for the krill resource.

This study focused on a relatively small nearshore region of Livingston Island, and it is difficult to say whether these results are applicable to other shallow, nearshore areas along the South Shetland Islands, Antarctic Peninsula, or elsewhere in the world. Acoustic surveys of krill backscatter further south along the Antarctic peninsula (Marguerite Bay) have also observed higher levels of backscatter in the shallower, nearshore regions (Lawson et al. 2004) that have been related to the distribution or abundance of at least one krill predator (Friedlaender et al. 2006), so the results of this study corroborate surveys in other areas conducted by larger vessels (but not in as shallow a region as this study).

The nearshore surveys were conducted over a very small area (hundreds of square kilometres) and very short time scale (days) over six of eight consecutive years. Because of these spatial and temporal sampling constraints, these data are susceptible to biases associated with short-term or small-scale phenomena that would be "smoothed out" by a larger area or longer survey period (Warren et al. 2009). Potential disturbances that may affect the nearshore survey results include mesoscale meteorological events such as low-pressure systems or storms passing through the region or possibly even the difference that a spring or neap tide might have on the ecosystem close to shore. Some of these events (particularly storms) are frequent occurrences in the study area, so these data may reflect some of these biases. However, the data from the nearshore survey during 2005 did not exhibit the same reduction in krill biomass that Warren et al. (2009) observed in the waters immediately adjacent to the nearshore survey area during the same time period, suggesting that the nearshore areas may be resistant to the effects of these submesoscale perturbations.

This study demonstrates that acoustic surveys conducted from instrumented small craft can augment larger-vessel surveys and extend the multidisciplinary investigations into waters commonly inaccessible, but ecologically important.

Results show that krill biomass can be as high or higher in some nearshore areas, possibly influencing the sites and success of penguin and seal rookeries, and should be considered in krill-fishery management schemes utilizing small-scale management units (Hewitt et al. 2004*b*). During the austral summer months, many of these highly productive nearshore areas are preferred foraging areas for large populations of penguins and Antarctic fur seals (Croll and Tershy 1998; Croll et al. 2006) and commercial fishing activities (Jones and Ramm 2004).

When rearing their chicks and pups on land, adult penguins and fur seals need a reliable food resource in the nearshore areas to assure reproductive success. Without adequate prey nearshore, the land-breeding krill predators must forage on other species, or further offshore, consequently jeopardizing the health of their offspring and themselves. Also, it should be noted that even in times of large nearshore krill abundance, the land-breeding predators might switch preferred prey. For example, Miller and Trivelpiece (2008) found that in 2004, the year with the second highest observed nearshore krill biomass density, the chinstrap penguins at Livingston Island preferentially foraged on fish instead of krill. The reason for this switch is unknown but may be due to increased accessibility of more nutritious prey. Increased competition between seals, penguins, and man for the nearshore krill resource, especially during the austral summer months, could have a large impact on the recruitment success of these land-based krill predators. Consequently, the biomass of krill within the foraging ranges of breeding penguins and seals should be routinely surveyed and considered when developing small-scale units, both in space and time, for managing the krill resource.

Acknowledgements

The financial and logistical support of the US AMLR Program has made this work possible, and Rennie Holt, its Director, is thanked heartily. During 2001, J.D.W. joined this research as an Office of Naval Research postdoctoral fellow (grant no. N00014-01-1-0166) with D.A.D. at NOAA's Southwest Fisheries Science Center (SWFSC). The 2005–2007 field seasons were supported by the National Science Foundation's Office of Polar Programs (grants OPP-0338196 and OPP-0633939) and the Advanced Survey Technologies group (AST) at SWFSC. The captains of R/V *Ernest*, Adam Jenkins (2000, 2002, 2004) and Steve Sessions (2005, 2006, and 2007), allowed these data to be safely collected in a wide variety of conditions. D.A.D.'s concept for R/V *Ernest I* was cleverly designed and skillfully built by Leif Knutsen (Big Foot Marine). Derek Needham and Steve Sessions assisted with the design and construction of R/V *Ernest II*. The Captain and crew of R/V *Yuzhmorgeologiya* provided a vigilant guard and reliable support for the small-boat operations. The scientists aboard the R/V *Yuzhmorgeologiya* provided great company and high-quality acoustic (A. Cossio, J. Emery, R. Hewitt, and C. Reiss), hydrographic and meteorologic (D. Needham, M. Prowse, M. Soule, and M. van den Berg), and zooplankton (V. Loeb and the zooplankton sampling teams) data. B. Cobb, B. Parker, D. Krause, and R. Haner of AMLR and J. Evans of NSF/Raytheon Polar Services provided logistical support for our land-based operations. Finally, this work was facilitated by the AMLR Cape Shirreff field camp personnel (led by R. Holt, M. Goebel, and W. Trivelpiece), who graciously welcomed, fed, and sheltered us during the small-boat operations.

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Population structure of lake trout (*Salvelinus namaycush*) in a large glacial-fed lake inferred from microsatellite DNA and morphological analysis

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Abstract: Understanding the structure of intraspecific genetic and morphological diversity within and across habitats is a fundamental aspect of biodiversity research with conservation value. Atlin Lake is the largest lake in British Columbia, Canada, and contains relatively pristine populations of lake trout (*Salvelinus namaycush*) that are key components of the lake's fish community and local fisheries. Lake trout from Atlin Lake were examined for genetic and phenotypic variation using eight microsatellite DNA loci, body form, and colouration. Genetic assays were also conducted on lake trout from the adjoining Tagish Lake and from 17 other localities to provide spatial context for the variation within Atlin Lake. The genetic data suggested that there were three genetic subpopulations within the Atlin–Tagish lake system. Morphological analysis identified two morphological groups of lake trout within Atlin Lake. Genetic and morphological groupings in Atlin Lake were not associated with each other. A mixed-stock analysis of samples collected from Atlin Lake commercial and recreational fisheries suggested that all genetic subpopulations contributed to the fishery and that there was some contribution from fish originating from within Tagish Lake.

Résumé : La compréhension de la structure de la diversité intraspécifique génétique et morphologique au sein des habitats et entre les habitats est une composante essentielle de la recherche sur la biodiversité reliée à la conservation. Le lac Atlin est le plus grand lac de la Colombie-Britannique, Canada, et il contient des populations de touladis (*Salvelinus namaycush*) encore relativement dans leur état originel qui forment une composante essentielle de la communauté de poissons du lac et de la pêche locale. Nous avons examiné la variation génétique et phénotypique des touladis du lac Atlin à l'étude de huit locus microsatellites d'ADN, de la forme corporelle et de la coloration. Nous avons aussi procédé à des analyses génétiques des touladis du lac Tagish voisin et de 17 autres localités pour obtenir un contexte spatial pour étudier la variation au sein du lac Atlin. Les données génétiques indiquent qu'il existe trois sous-populations génétiques dans le réseau des lacs Atlin et Tagish. L'analyse morphologique a permis d'identifier deux groupes morphologiques de touladis dans le lac Atlin. Les regroupements génétiques et morphologiques dans le lac Atlin ne sont pas associés l'un à l'autre. Une analyse de stock mixte d'échantillons récoltés dans les pêches commerciales et sportives au lac Atlin montre que toutes les sous-populations génétiques contribuent à la pêche et qu'il y a une certaine contribution des poissons provenant du lac Tagish.

[Traduit par la Rédaction]

Introduction

The origin, extent, and patterns of genetic diversity within species are central issues for evolutionary biologists (Futuyma 1997) and are increasingly important for conservation because genetic diversity can influence the persistence of populations (Frankham et al. 2002). Consequently, there is a growing need to study genetic diversity of contemporary populations to help understand how population viability might change in response to environmental fluctuations (Schwartz et al. 2007). One aspect of genetic variation is population subdivision, the partitioning of a species into two or more independent or semi-independent genetic subpopulations that may exist in the same or different habitats (e.g., Waples and Gaggiotti 2006).

In many fishes, including salmonids (salmon, trout, char, whitefish, and grayling), assays of population distinctiveness have been employed in a variety of conservation contexts such as separating native from introduced fish (e.g., Taylor et al. 2007) or to identify distinct populations to facilitate population-specific management initiatives (Gunn et al. 2003). As dominant predators of north-temperate freshwater lakes in the Nearctic, lake trout (*Salvelinus namaycush*) are

Received 12 April 2009. Accepted 22 March 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 25 June 2010.
J21158

Paper handled by Associate Editor Michael Hansen.

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doi:10.1139/F10-054

important members of aquatic communities across their range. Lake trout are exploited, to varying degrees, in traditional, commercial, and recreational fisheries and declines in abundance and loss of specific populations are occurring in a number of areas (Guinand et al. 2003; Gunn et al. 2003). In addition, lake trout populations are vulnerable to other human activities, including the introduction of non-native species (Smith and Tibbles 1980; Guinand et al. 2003; Dextrase and Mandrak 2006). In an effort to better manage exploitation-based and environment-based stressors on lake trout populations, the species has been the subject of a number of studies to resolve population distinctiveness. For instance, the study of sympatric and allopatric lake trout populations has resolved genetic differences in survival, morphology, early development rate, physiology, allozymes, and microsatellite allele frequencies (Brown et al. 1981; Ihssen et al. 1988; Guinand et al. 2003). Genetic "inventories" may help to identify populations of conservation significance; populations with low levels of genetic variability might be compromised in their ability to persist in current or changing environments, and populations with high levels of diversity might be of great value for opposite reasons (Frankham et al. 2002; Piller et al. 2005).

Many north-temperate fish species consist of two or more major genetic lineages thought to have arisen from divergence during isolation in distinct glacial refugia — divergence perhaps enhanced by postglacial adaptation to their present environments (Bernatchez and Wilson 1998; Power 2002). The many episodes of isolation while in separate refugia followed by secondary contact between divergent lineages have probably contributed to the tremendous morphological, ecological, and genetic variability observed within lake trout that makes them an excellent model for studying evolutionary process of divergence (Magnan et al. 2002).

Atlin Lake is located in northwestern British Columbia (BC), Canada, and is the province's largest natural lake ecosystem, with a surface area of 792 km^2. Atlin Lake forms one of the headwater lakes of the Yukon River system and interconnects with Tagish Lake. Lake trout are thought to have colonized this area from both the Nahanni and Beringian refugia using freshwater dispersal corridors (no anadromous populations of lake trout are known) beginning about 10 000 years ago (Wilson and Hebert 1998). In addition, because of its large size and diversity of habitats, Atlin Lake has the potential to contain multiple genetically distinct populations of lake trout. There have, in fact, been anecdotal accounts of different lake trout phenotypes (based on observations of shape, size, and colour) occurring in different parts of the lake (N. DeGraaf, Taku River Tlingit First Nation, Atlin, BC, unpublished data). In addition to the importance of lake trout from Atlin Lake to the local community, the lake is of interest to a broader understanding of lake trout biology and conservation. This is because relatively few populations in the western part of the geographic range of lake trout have been examined in detail genetically, especially for pristine areas such as Atlin Lake. Furthermore, there are longstanding accounts of variation in lake trout in body shape and colour, variability initially thought to be restricted to the Laurentian Great Lakes (Dehring et al. 1981; Gunn et al. 2003; Bronte and Moore 2007). Subsequent investigations have found morphological variants in other northern lakes (Blackie et al. 2003; Alfonso 2004; Zimmerman et al. 2006). How geographically widespread such variants are and how they originate or are maintained in their respective environments, however, remain unknown.

Despite the fact that lake trout from different areas of Atlin Lake appear to spawn at the same time of year, variation in food source, water depth, and water turbidity may promote local differentiation. If more than one morphotype exists in Atlin Lake, it would be important to determine if these morphotypes signal genetically distinct populations such that a better understanding of the basis of within-lake biodiversity can be obtained (Keeley et al. 2007). For instance, should distinct morphotypes be a purely phenotypic response to variable environments within the lake, then management might focus on maintaining a minimum population size and a diversity of habitats across the entire lake so that this plasticity is sustained. Alternatively, if morphotypes are associated with genetically distinct populations of lake trout, then these morphotypes provide a means to monitor genetic diversity and efforts should be directed towards understanding the biological processes that sustain such genetic variation (spatial or ecological segregation) such that a plan for the maintenance of within-lake biodiversity can be designed. Maintenance of such intraspecific genetic biodiversity is considered important for the long-term persistence of species (Bowen 1999; Allendorf and Luikart 2007) and their productivity within specific habitats (Hilborn et al. 2003), as well as for ecosystems as a whole (Booth and Grime 2003; Reusch et al. 2005). Further, a common problem in fisheries management is that sometimes the management of only fish occurs when it should include assessment of fishers and their effort (Hilborn 2007). Lake trout in Atlin Lake are exploited in commercial and recreational fisheries but have not been subjected to fish stocking programmes. Consequently, if Atlin Lake contains genetically distinct subpopulations of lake trout, an understanding of the relative levels of exploitation of each component through mixed-stock fishery analysis would help guide a conservation plan for each subpopulation integrated across the whole lake.

To contribute to a greater understanding of the structure of diversity in Atlin Lake *S. namaycush* and provide guidelines for more effective management and conservation, we conducted a survey of microsatellite DNA and morphological variation in collections of lake trout from Atlin Lake, as well as from several other localities in BC and Yukon. Although investigations on lake trout from Lake Mistassini, Québec, and Great Bear Lake, Northwest Territories, are recent exceptions (Blackie et al. 2003; Zimmerman et al. 2007), the vast majority of data on variability in lake trout have been collected for highly perturbed populations of lake trout in the Laurentian Great Lakes. Consequently, our data contribute to much needed comparative data from relatively understudied and less perturbed northwestern ecosystems.

Materials and methods

Sample collections

We obtained lake trout tissue samples from adult fish that were collected between 2000 and 2006 from 19 lakes in BC, Yukon, and Northwest Territories using a variety of gillnet gear (1.5- to 3-inch mesh sizes) that captured fish ranging from 192 to 887 mm fork length. The lakes sampled varied in surface area (determined from topographic maps) from 1.1 to 792 km^2 (Table 1; Fig. 1). Three lakes (Trapper, Arctic, and Muncho) had sample sizes <10 individuals, and we used them only in general descriptions of variability. We sampled two lakes from the upper Yukon River watershed in northwestern BC and adjacent Yukon Territory (Atlin and Tagish lakes) more extensively. These two lakes are interconnected by the Atlin River, are very large (>300 km^2 in surface area), and are characterized by very low levels of development and, presumably, human disturbance. For about one-half of its length, Atlin Lake is surrounded by Atlin Provincial Park. In Atlin Lake, we collected fish during general lake surveys (N = 186) using small-mesh gillnets deployed at 1 km intervals for 1 h, as well as by sampling catches landed at the Great Northern Fish Company (GNFC, N = 101). We also sampled tissue from adults obtained in the recreational fishery (N = 33). Fish from Atlin Lake were weighed and measured for fork length, and adipose fin tissue was removed and stored in 95% ethanol. We calculated condition factor of each fish as (weight (in grams) × 100)/length^3 (in cm). In 2005 and 2006, samples from the Atlin Lake survey were photographed for morphological and colouration analyses (see below). In Tagish Lake, we collected adult fish using gillnets from known spawning reefs (N = 175), as well as from nonspawning areas (N = 52), analogous to the whole-lake surveys conducted in Atlin Lake.

Genetic data collection and analyses

We extracted total genomic DNA using the QIAgen DNeasy kit system, and DNA was precipitated and resuspended in Tris–EDTA (pH 8.0) buffer and then stored at –20 °C. Polymerase chain reactions (PCR) were performed across eight loci in 10 μL volumes in MJ PTC 100 thermal cyclers using fluorescently labeled primers and assayed on a Beckman-Coulter CEQ 8000 automated capillary sequencer/genotyper (Supplemental Table S1[2]). The loci used were Sco2, Sco102, Sco107, Sco19, Sco215, Sfo18, Ssa197, and Smm22 (Supplemental Table S1[2]). We routinely re-ran individual samples across all loci to check for consistency of scoring; in no case did these re-runs produce results that differed from the original analyses.

We tested for departures from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium using Fisher's exact test in which P values were estimated using the Markov chain method as implemented in GENEPOP (version 3.1; Raymond and Rousset 1995). We used the sequential Bonferroni correction method (Rice 1989) to adjust critical type-I error rates when making multiple simultaneous hypothesis tests. For tests within populations, we adjusted alpha levels sequentially by the number of loci for tests of HWE and by the number of pairwise comparisons between loci for tests of linkage disequilibrium. Basic descriptive statistics of sample size (N), number of alleles (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) were compiled using TFPGA (version 3.2; Miller 1997), whereas allelic richness (A_R) was determined using FSTAT (version 2.9; Goudet 2001). Weir and Cockerham's (1984) F_{ST} (θ) values were calculated using ARLEQUIN (version 3.0; Excoffier et al. 2005) to test population differentiation via pairwise comparisons. There was no evidence from permutation tests in SPAGeDi (Hardy and Vekemans 2002) that mutation contributed to population differentiation, so we limited our genetic distance inferences to those based on F_{ST}. Next, we used PCAgen (Goudet 1999) to conduct a principal component analysis (PCA) on allele frequency data to visualize genetic differentiation among the sample localities. Microsatellite variation was also partitioned into its components by performing an analysis of molecular variance (AMOVA) following Excoffier et al. (1992) and as implemented in ARLEQUIN. Different hierarchical arrangements of samples such as watershed groupings, PCA groupings, and Atlin Lake and Tagish Lake subpopulations (see below) were tested.

We tested for a correlation between geographic distance and genetic distance (F_{ST}) and its significance to determine if the observed genetic structure could be explained by the isolation-by-distance model (IBD; Slatkin 1993). We used TFPGA to perform the Mantel test (Mantel 1967), and significance of any correlation was determined using the default setting of 999 matrix permutations. We used Google Earth (http://earth.google.com/download-earth.html) to calculate the distances between localities.

We adopted several approaches to estimating the population structure of lake trout within Atlin and Tagish lakes. First, the number of subpopulations, K, within Atlin Lake and Tagish Lake was estimated by employing the program STRUCTURE (version 2.2; Pritchard et al. 2000). Because Atlin and Tagish lakes are interconnected by the Atlin River and lake trout are present in this waterway, we did not conduct STRUCTURE analyses separately by lake. A Bayesian clustering algorithm, Markov chain Monte Carlo based approach assumes a model of K subpopulations, characterized by allele frequencies at each locus and assigns individuals probabilistically to each of these subpopulations. This analysis then uses a likelihood approach to determine the most probable number of K subpopulations using a general genetic inheritance model to minimize Hardy–Weinberg and linkage disequilibrium within the subpopulations. We ran multiple trials following Evanno et al. (2005), with a "burn-in" period of 10 000 iterations followed by 10 000 iterations to estimate K and subpopulation membership; longer runs with more iterations did not alter the basic results. Initial analyses examining values of K from 1 to 30 suggested that the true value of K was ~5 within Atlin and Tagish lakes. Consequently, we conducted more detailed simulations employing the admixture model with correlated allele frequencies at each K from 1 to 8 using a burn-in of 50 000 iterations followed by 450 000 iterations and replicated each five times per K value. The model of K with the highest log-likelihood value was chosen as the most probable level of population subdivision. We did not employ the ad hoc statistic, ΔK (Evanno et al. 2005), because of the generally low levels of subdivision resolved where this statistic performs poorly at resolving the true K (Waples and Gaggiotti 2006). After the most likely number of subpopulations was deter-

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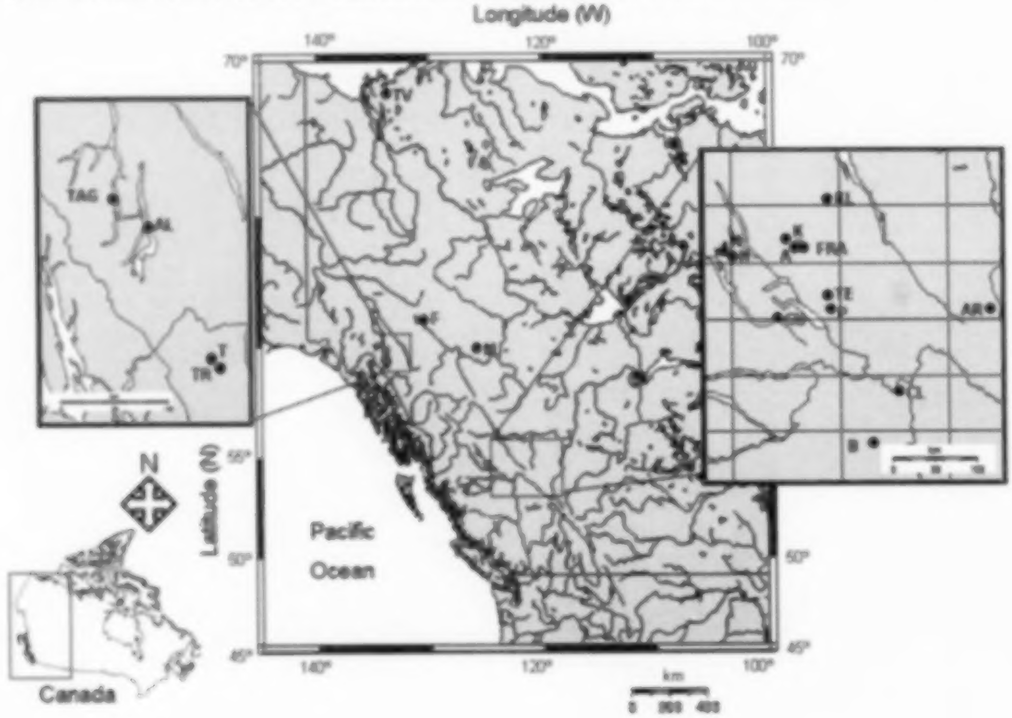
[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Table 1. Sample sites, sample size (*N*), locations, watersheds, elevations, and surface area of lakes sampled for lake trout (*Salvelinus namaycush*).

Lake	Location	*N*	Latitude (N)	Longitude (W)	Watershed	Elevation (m)	Area (km²)
1. Atlin (AL)	BC/Yukon	186	59°29′57.14″	133°44′12.07″	Yukon	886	792
2. Tasemerie (T)	BC	35	58°20′47.47″	132°19′14.49″	Taku	1270	~57
3. Tagish (TAG)	BC/Yukon	227	59°46′21.87″	134°14′29.46″	Yukon	1109	354.6
4. Trapper (TR)	BC	9	58°27′27″	132°37′32″	Taku	1457	~24
5. Muncho (M)	BC	6	58°59′38″	125°46′57″	Mackenzie	1494	~50
6. Frances (F)	Yukon	19	61°23′	129°35′	Mackenzie	804	99.4
7. Travaillant (TV)	NWT	17	69°39′16.95″	131°51′03.75″	Mackenzie	27	~102
8. Bednesti (B)	BC	25	53°22′41″	123°51′44″	Fraser	1201	2.61
9. Hautete (H)	BC	32	55°11′14″	126°8′50″	Fraser	989	2.3
10. Tezzeron (TE)	BC	23	54°42′52″	124°27′59″	Fraser	910	78.1
11. Arctic (AR)	BC	8	54°25′17″	121°40′36″	Fraser	1327	1.1
12. Cluculz (CL)	BC	24	53°52′23″	123°34′20″	Fraser	860	19.9
13. Fraser (FRA)	BC	27	55°4′56″	124°45′3″	Fraser	880	54.6
14. Nakinlerak (K)	BC	36	55°14′52.512″	125°14′54.134″	Fraser	1092	7.3
15. Natowite (N)	BC	32	55°5′11″	125°56′46″	Fraser	938	16
16. Cunningham (CU)	BC	30	54°35′31″	125°19′20″	Fraser	863	29.9
17. Airline (A)	BC	28	55°7′43″	125°3′47″	Fraser	839	4.5
18. Klawli (KL)	BC	28	55°21′0″	124°21′43″	Fraser	1006	4.6
19. Pinchi (P)	BC	26	54°36′43″	124°24′28″	Fraser	830	55.5

Note: BC, British Columbia; NWT, Northwest Territories.

Fig. 1. Locations in western Canada where lake trout (*Salvelinus namaycush*) were collected in this study. Population codes are given in Table 1. Inset at the top left shows Atlin and Tagish lakes in northwestern British Columbia and Yukon; inset on the right shows the upper Fraser River, British Columbia; inset at the bottom left shows the whole study region in relation to Canada.



mined, each fish was assigned to the subpopulation for which its inferred ancestry coefficient, *q*, was at least 0.5 (i.e., the proportional contribution to its genome across the eight loci from a specific subpopulation was estimated to constitute at least 0.5; Pritchard et al. 2000).

We partitioned Atlin Lake into four large geographic regions that corresponded to areas that are partially isolated from each other or that had distinct limnological conditions: north arm (northernmost 50 km of lake), central arm (next 50 km of lake), west arm (distinct restrictions between this area and central and south arms), and south arm (southernmost 25 km of lake headed by the Llewellyn Glacier field). Contingency tests were performed to assess statistical significance of differences in the frequencies of the STRUCTURE-generated genetic subpopulations among these geographic regions within Atlin Lake using PAST, a general spreadsheet-based package of statistical analyses (Hammer et al. 2001). Similar tests were also performed to assess whether the genetic subpopulations within Tagish Lake were associated with distinct spawning locations.

We next calculated individual pairwise identity indices (Mathieu et al. 1990) in the Atlin Lake and Tagish Lake subpopulations identified by STRUCTURE with IDENTIX (Belkhir et al. 2002). The identity index varies from 0 to 1.0 and provides a measure of relatedness because closely related individuals are more likely to produce homozygous offspring and IDENTIX estimates the expected proportion of loci that are homozygous in the offspring of any pair of individuals. IDENTIX also implements a permutation test of the null hypothesis of no relatedness by comparing the distribution of the observed pairwise relatedness coefficients in a population sample with its null expectation. The identity values were calculated because the sampling was not based on actual spawning locations, at least in Atlin Lake, and examining this estimate of individual relatedness may resolve finer patterns of genetic differentiation especially because kin groups have been resolved in other char at postjuvenile life stages (Fraser et al. 2005).

The composition of baseline populations defined by genetic data for Atlin and Tagish lakes and for use in subsequent fisheries mixture analyses was determined using STRUCTURE by identifying the *K* most likely subpopulations for both Atlin and Tagish lakes' survey samples and assigning each individual to its most likely population (i.e., $q > 0.5$) as described above. These baseline subpopulations were used in conjunction with genetic mixture analysis to determine if the commercial and recreational fisheries in Atlin Lake were sampling proportionately from each population using the Bayesian approach to mixed-stock analysis implemented in ONCOR (Anderson et al. 2008). This analysis utilizes the conditional maximum likelihood algorithm to estimate mixture proportions (Miller 1987) and the Rannala and Mountain (1997) method for estimating the probability of observing a genotype in series of baseline populations, each of which was characterized across a number of genetic loci. We estimated the robustness of our analyses in two ways. First, 95% confidence intervals were generated by bootstrap resampling with replacement in both the mixture samples (across individuals) and the baseline samples (alleles across genotypes) during 5000 replicate analyses. Second, we produced simulated mixtures ($N = 200$) in which we fixed the proportional contribution of each subpopulation in turn to 1.0 and then estimated the mixture proportions for all baseline subpopulations contributing to the simulated mixtures. In this case, "perfect" performance of the mixture analysis would return an estimated proportional contribution for each subpopulation of 1.0. Initially, this analysis compared the commercial and angling samples with all 19 lakes sampled to determine the general performance of our data and ONCOR (i.e., no contributions from lakes other than Atlin and Tagish should be observed). Next, commercial and recreational fishing samples were analysed, both separately and in a combined analysis, to assess the contributions of the various genetic subpopulations from Atlin and Tagish lakes.

Morphological analyses

We photographed individual fish from Atlin Lake using a digital camera with a measuring board as a consistent background for light standardization. Body shape was measured with the aid of TPSDIG software (Rohlf 1997). The digital images were imported into TPSDIG and *x* and *y* coordinates were established using 16 (each with an *x* and *y* coordinate) landmarks to measure the shape of each fish (Zimmerman et al. 2006; Bronte and Moore 2007; Supplemental Fig. S1[2]). These data were then imported into a companion program TPSRELW (Rohlf 1997) that centers, scales, and aligns the coordinates using the Procrustes method and calculates an average configuration to describe the consensus body shape among a sample of fish. The program then compares each set of coordinates with the consensus body shape using thin-plate spline analysis (Bookstein 1991). The method produces a value for each fish that represents its deviation at each *x* and *y* coordinate from the consensus shape, generating 32 such deviation scores for each fish (*x*, *y* coordinates across 16 landmarks). The TPSDIG software was also used to measure head depth, midbody depth, and caudal peduncle depth and to confirm body length, which had been measured in the field. To account for variation in body size related (isometric) differences, head depth, midbody depth, and caudal peduncle depth were size-corrected to the overall sample mean fork length (521 mm) by employing the residuals from the linear relationship between fork length and individual trait in subsequent statistical tests (Thorpe 1976). These latter three measurements, in combination with condition factor and the 32 deviation scores from the landmark analysis, resulted in 36 body shape measurements for each fish.

We quantified body colouration, or more accurately brightness, of each lake trout image with the aid of Image J software (version 1.32j; http://rsb.info.nih.gov/ij) following the methodology of Zimmerman et al. (2006). All images were converted to black and white and the measurements were taken on a 0 (black) to 256 (white) scale and averaged for a total overall "brightness factor". Brightness was measured at six positions along the lateral surface of each fish as defined by Zimmerman et al. (2006): midoperculum, two equidistant points along the body above the lateral line, two below the lateral line, and one at the midpoint of the caudal peduncle. We performed ANOVAs to assess the level of brightness differentiation among (*i*) the genetic subpopulations resolved via STRUCTURE, (*ii*) body shape based morphological clusters (see below), and (*iii*) within-lake geographical units.

To summarize morphology, condition factor, and brightness into a measure of overall phenotypic variation, we conducted principal component analysis (PCA) on the correlation matrix among all variables using PAST. To determine the number of phenotypic clusters that could be resolved among all lake trout, a model-based clustering method was employed without a priori designation of populations using the program MCLUST as implemented within the R Statistical Software Project (Fraley and Raftery 2003; R Development Core Team 2008). The method fits the observed frequency distribution of PCA scores obtained from the analysis above to alternative models of structure in terms of the number, shape, and variability within phenotypic clusters. In the first model, a single morphological cluster is assumed to best represent the data. In subsequent models, two or more (up to eight in our case) clusters are assumed to exist. The model with the highest Bayesian information criterion (BIC) is selected as the model best describing morphological variation, with differences in BIC values between alternative models that are greater than 6 indicating "strong" evidence and those greater than 10 indicating "very strong" evidence in favour of the model with the highest BIC value (Fraley and Raftery 2003). The MCLUST analysis includes a function allowing classification of individual observations (fish) to the resolved morphological groups.

We conducted a discriminant function analysis (DFA) with jackknifing in PAST to measure the reliability of classification of individual fish to morphological groups resolved in the MCLUST analysis. A contingency test was also performed using PAST to determine (*i*) if the morphological groups resolved above were associated with the four geographic regions in Atlin Lake and (*ii*) if there was a relationship between the STRUCTURE-defined genetic subpopulations and the morphological clusters. Finally, we estimated the number of genetic groups within the sample of fish examined morphologically using STRUCTURE as detailed above, testing among potential *K* values of 1–5.

Results

Intrapopulation genetic variation

We assayed microsatellite variation across 818 individuals at eight loci. The number of alleles ranged from four (Sco102) to 30 (Smm22) across all populations, with an average of 14.8 alleles per locus for the entire study region and an average of 5.3 alleles per locus per lake (Supplemental Tables S1 and S2[2]). Observed heterozygosity ranged from 0.17 to 0.91, with an average of 0.51 across all loci and populations (Supplemental Table S2[2]). The two most variable loci were Smm22 and Ssa197, with 30 and 24 alleles globally, respectively. Fourteen out of 152 (8 loci × 19 populations) tests showed statistically significant deviations from HWE, and all were heterozygote deficiencies. Seven such deficiencies were from Atlin Lake (four loci) and Tagish Lake (three loci); the remaining samples were largely in HWE. Tests for linkage disequilibrium resulted in none of the 532 tests being significant. Consequently, we considered each locus to represent an independent measure of genetic variation and divergence.

The level of variation within populations varied greatly. Expected heterozygosity over all eight loci ranged from a low of 0.30 (Bednesti Lake) to a high of 0.75 (Atlin Lake) (Supplemental Table S2[2]). Fourteen populations were found to have at least one of the eight loci fixed, with Sco102 being the most commonly fixed locus. No more than two loci, however, were fixed in any one population. Between watersheds, there were no fixed allele differences among the eight microsatellites studied; however, there were several alleles found to be unique to individual watersheds, especially within the Yukon and Fraser rivers' watersheds. There was a strong, positive correlation between lake surface area and allelic richness ($r = 0.68$, $P = 0.001$).

Population structure among lakes

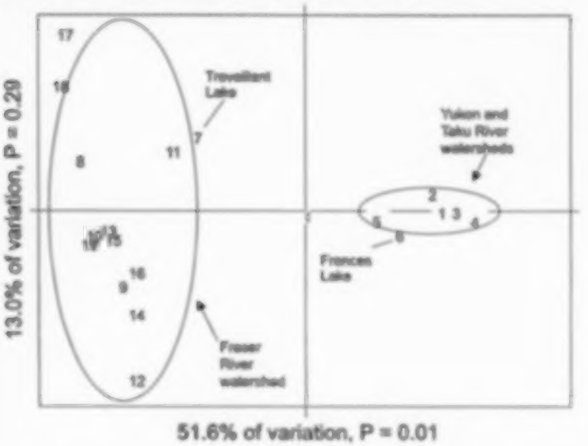
There were 120 pairwise comparisons of F_{ST} calculated across the eight loci between populations with sample sizes greater than 10 ($N = 16$), and all but two of these were significant at $\alpha = 0.05$ (Supplemental Table S3[2]). The average F_{ST} across all comparisons was 0.258 and ranged from 0.009 to 0.616. A principal component analysis (PCA) of allele frequency for all lakes revealed two groupings, with PCA 1 being the major (and only significant, $P < 0.01$) axis of differentiation between groupings (Fig. 2). Lakes in the Fraser River watershed and Travaillant Lake (Mackenzie River, Northwest Territories) formed one group that was separate from the remaining samples (Yukon, northwestern BC). We next examined genetic differentiation by grouping the various populations by watershed; AMOVA indicated that variation among the watershed groups (19.6%, $P < 0.001$) exceeded that among populations within watersheds (13.8%, $P < 0.001$; Table 2). Further tests were conducted based on the groups suggested by the principal component analysis (Fraser River – Travaillant Lake vs. all others; Fig. 2). Here, there was a considerable narrowing of the difference between the two PCA groups (3.3%, $P < 0.001$), as well as that among populations within PCA groups (6.0%, $P < 0.001$).

When all populations were included, a test for IBD was not significant ($r = 0.27$, $P = 0.09$). The populations were then grouped according to the two PCA clusters and tested for IBD within each group, which was also not significant within either group ($r = 0.34$, $P = 0.25$, and $r = 0.24$, $P = 0.22$, respectively). When lakes from the Fraser River watershed were analyzed separately, however, we detected a significant pattern of IBD ($r = 0.47$, $P = 0.025$).

Population structure within lakes

Four hundred and thirteen individuals from Atlin and Tagish lakes were genotyped at eight microsatellite loci: 186 from Atlin Lake and 227 from Tagish Lake. The most variable loci were Smm22 and Ssa197, with 26 and 18 alleles, respectively. STRUCTURE analysis indicated the presence of genetic substructure, with three clusters (ATL-A, $N = 94$ fish; ATL-B, $N = 123$ fish; ATL-C, $N = 196$ fish) being the most likely number of subpopulations within these interconnected lakes (Supplemental Table S4[2]). When we pooled individual fish by membership within a subpopulation, only three of the 24 (eight loci × three subpopula-

Fig. 2. Principal component analysis of allele frequency variation at eight microsatellite loci among all sample lakes. The numbers correspond to the population codes listed in Table 1.



tions) tests showed statistically significant HWE deviations. Tests for linkage disequilibrium resulted in none of the 84 pairwise tests being significant. Within any of the three subpopulations, the number of alleles ranged from three (Sco102, subpopulations ATL-A and ATL-B) to 24 (Smm22, subpopulation ATL-A), with the three subpopulations averaging 10.6, 10.1, and 11.8 alleles, respectively (Supplemental Table S5[2]). Observed heterozygosity ranged from 0.18 (subpopulations ATL-A and ATL-B, Sco102) to ~0.90 (subpopulations ATL-A and ATL-C, Smm22), with an average of 0.65 across all loci and populations (Supplemental Table S5[2]).

Estimates of F_{ST} between the three subpopulations averaged 0.022 and ranged from 0.005 between subpopulations ATL-A and ATL-C to 0.027 between ATL-A and ATL-B to 0.034 between ATL-B and ATL-C (all $P < 0.001$). Pairwise identity values averaged 0.25, 0.38, and 0.27 in subpopulations ATL-A, ATL-B, and ATL-C, respectively, and none was significantly different from that expected under random mating within subpopulations (all $P > 0.20$).

Contingency tests failed to find any geographic pattern in the distribution of the three genetic groupings across the four areas of Atlin Lake ($P = 0.25$; Fig. 3). In contrast to Atlin Lake, however, there was a highly nonrandom association between membership of individual fish in one of the three genetic subpopulations and area of capture (spawning bed) within Tagish Lake ($P < 0.0001$; Fig. 3). Furthermore, there was a strong difference in the frequency of the three genetic subpopulations between Atlin and Tagish lakes (contingency test, $P < 0.0001$; Fig. 3). In particular, Tagish Lake possessed a higher frequency of genetic subpopulation ATL-B than did Atlin Lake. We performed an analysis of molecular variation by pooling all STRUCTURE-generated subpopulations from Atlin and Tagish lakes by geographic region within lake. The AMOVA indicated that only a small, but significant, percentage of the total variation found was attributable to differences between lakes (1.2%, $P = 0.04$), but slightly more was attributable to differences among regions within lakes (2.3%, $P < 0.001$), and most variation was attributable to differences among individual fish within subpopulations (96.6%, $P < 0.001$).

Mixed-stock fisheries analysis

Initial comparisons of lake trout commercial and angling samples ($N = 134$) from Atlin Lake with baseline samples from all 19 lakes in this study estimated that 79.6% of these samples were from Atlin Lake, while 20.4% originated from Tagish Lake. As expected, no other lakes were identified as contributing to the Atlin Lake fisheries. Lake trout from commercial fisheries and those caught by local anglers were compared with the three genetic subpopulations identified both in Atlin and Tagish lakes. Commercial fisheries appeared to draw most heavily from subpopulation ATL-C (82.7%), followed by subpopulation ATL-B (15.1%), and finally from subpopulation ATL-A (2.2%) (Table 3). The angling catch also appeared to sample most heavily from subpopulation ATL-C, followed by ATL-B, but few fish from ATL-A were apparently exploited (Table 3). When we partitioned the baseline and commercial samples by lake, mixture analysis suggested that about 75% of all fish exploited originated from Atlin Lake (Table 3), with the remainder from Tagish Lake. The angling catch was even more dominated by fish from Atlin Lake (91% vs. 9%; Table 3). Finally, all samples were partitioned by lake region (Atlin) or spawning ground (Tagish). In this analysis, 75% of commercially exploited fish were estimated to have originated from Atlin region 4 and Tagish Lake region SW (Table 3). The angling catch was also dominated by fish from Atlin Lake region 4, but other Atlin Lake regions and Tagish Lake region DB also appeared to contribute to the recreational fishery (Table 3). Interestingly, both in commercial and angling analyses, Tagish Lake region W did not appear to contribute any fish (Table 3). Confidence intervals for real and simulated mixture analyses by genetic subpopulation and lake were largely nonoverlapping and associated with minimum baseline sample sizes of about 100 (Table 3). This was in marked contrast to the poor performance of mixture analyses conducted by region where simulated proportions were highly variable with broad confidence intervals that all included 0. A baseline sample size of at least 50 appears to be required for simulated mixtures to approach 1.0 with relatively narrow confidence limits (Table 3).

Morphological analysis

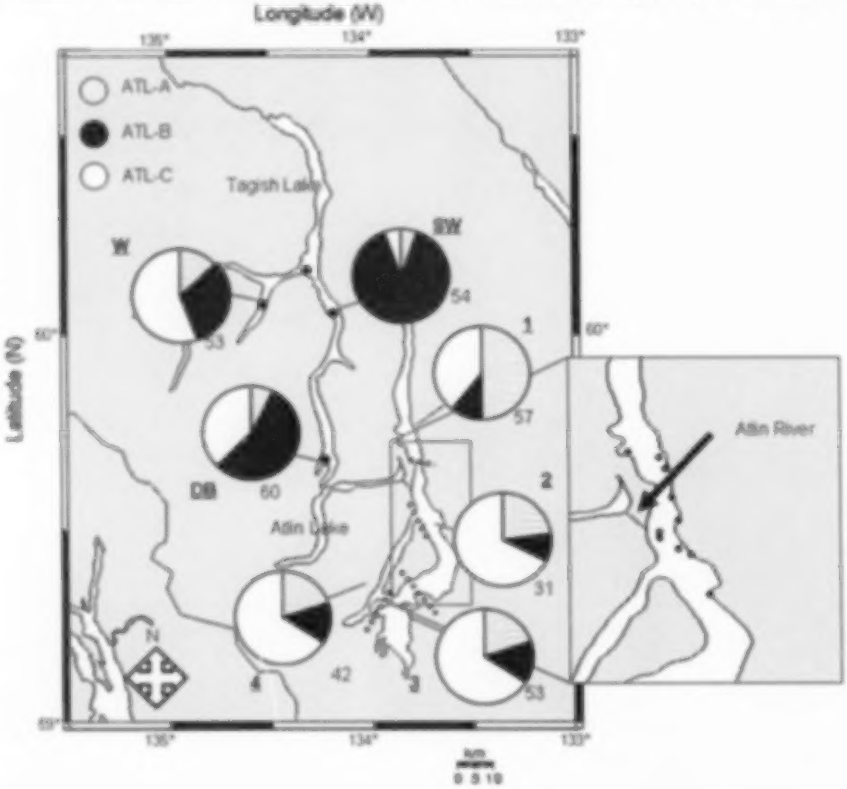
We examined 104 lake trout morphologically and in terms of body brightness. Two components in the PCA accounted for 56.2% of total variation and axes 1 and 2 (PC1 and PC2) made up 40.7% and 15.5% of the total variation, respectively (Supplemental Table S6[2]). Fish length was uncorrelated, with scores along PC1 or PC2 (both $P > 0.1$) suggesting the lack of an allometric effect on body shape differences. A model describing two distinct morphological clusters with unequal variances within them had the highest BIC of −1080 (Fig. 4). Alternative models had BIC values at least 20 points lower than the model describing two populations: MCLUST reported a BIC of −1110 for models with one morphological cluster and a minimum BIC of −1100 for models with three or more clusters. The separation of the two clusters was evident, however, only along PC2, which appeared to differentiate fish with long heads and

Table 2. Analysis of molecular variance within and among western lake trout (*Salvelinus namaycush*) populations based on allele frequency variation across eight microsatellite DNA loci.

	Yukon vs. Taku vs. Mackenzie vs. Fraser watersheds			Between PCA groups			Atlin vs. Tagish		
Variance component	$F_{statistic}$	% variation	P	$F_{statistic}$	% variation	P	$F_{statistic}$	% variation	P
Between regions or lakes	0.196	19.58	<0.001	0.149	14.97	<0.001	0.012	1.2	0.04
Among populations within regions or lakes	0.333	13.76	<0.001	0.137	11.64	<0.001	0.023	2.2	<0.001
Within populations among regions or lakes	0.171	66.66	<0.001	0.26602	73.40	<0.001	0.034	96.6	<0.001

Note: Comparisons between Atlin and Tagish lakes incorporated variation among three genetic subpopulations resolved within each lake, respectively.

Fig. 3. Map of the distribution of lake trout (*Salvelinus namaycush*) genetic subpopulations within Atlin and Tagish lakes according to STRUCTURE analysis. The bold dashed lines indicate the separation between geographical regions in Atlin Lake. Shading within each pie chart depicts the frequency of each of the three genetic subpopulations at that locality, with the sample size shown as the numeral to the right of each pie chart. Solid circles represent known or suspected spawning locations in Atlin and Tagish lakes (M. Connor, Taku River Tlingit First Nations Fisheries Department, unpublished data). Locality codes are given as underlined numbers for each of the four regions in Atlin Lake or abbreviations for the three spawning reefs in Tagish Lake (DB, Deep Bay; W, Windy Arm; SW, Squaw Point).



caudal regions from those with deeper heads and bodies overall (Supplemental Table S6[2]; Fig. 4). The five top-ranked positive coefficients along PC2 were for x1, x3, x4, x10, and x11 associated with head, eye, and caudal region landmarks (Supplemental Fig. S1 and Supplemental Table S6[2]). The most negative coefficients for PC2 were for x6, x7, x14, x15, and y5 associated with fin position and head landmarks (Supplemental Fig. S1 and Supplemental Table S6[2]). Condition factor and "brightness" score had low PC coefficients along PC2, typically in the order of five or six times lower than for the landmarks with the highest coefficients (Supplemental Table S6[2]). In general, fish that scored

Table 3. Genetic mixture analysis results for 101 commercial samples and 33 recreational angling samples of lake trout (*Salvelinus namaycush*) from Atlin Lake as generated using ONCOR (Anderson et al. 2008) and variation at eight microsatellite DNA loci.

		Sample mixture		
Group	N	Commercial	Angling	Simulated mixture
Genetic				
ATL-A	94	0.022 (0.000–0.110)	0.258 (0.034–0.462)	0.990 (0.971–1.000)
ATL-B	123	0.151 (0.053–0.267)	0.002 (0.000–0.262)	0.979 (0.954–0.999)
ATL-C	196	0.827 (0.688–0.924)	0.741 (0.456–0.927)	0.995 (0.977–1.000)
Lake				
Atlin Lake	186	0.773 (0.587–0.884)	0.908 (0.612–0.999)	0.953 (0.898–0.999)
Tagish Lake	227	0.227 (0.116–0.410)	0.092 (0.001–0.385)	0.964 (0.917–0.999)
Region				
Atlin Lake 1	18	0.068 (0.000, 0.193)	0.152 (0.000, 0.357)	0.225 (0.149–0.297)
Atlin Lake 2	21	0.121 (0.018, 0.276)	0.119 (0.000, 0.375)	0.284 (0.169–0.379)
Atlin Lake 3	30	0.001 (0.000, 0.184)	0.148 (0.000, 0.408)	0.361 (0.278–0.446)
Atlin Lake 4	56	0.503 (0.248, 0.606)	0.269 (0.000, 0.602)	0.630 (0.527–0.728)
Tagish Lake W	55	0.000 (0.000, 0.062)	0.000 (0.000, 0.006)	0.763 (0.665–0.848)
Tagish Lake SW	55	0.252 (0.044, 0.379)	0.080 (0.000, 0.370)	0.633 (0.545–0.697)
Tagish Lake DB	61	0.056 (0.000, 0.201)	0.233 (0.001, 0.457)	0.969 (0.921–0.999)

Note: Tagish Lake: W, Windy Arm; SW, Squaw Point; DB, Deep Bay. Mixture values represent the estimated contribution of each genetic subpopulation within Atlin and Tagish lakes, for lakes, and for geographic regions (Atlin Lake) or spawning localities (Tagish Lake) within lakes averaged (95% confidence intervals (CI) in parentheses) over 5000 bootstrap replicates. Subpopulations or lakes or regions with the highest average proportional contributions are indicated in bold. Simulated mixtures indicate average (95% CI) proportional contribution of each subpopulation or lake or region in simulated mixtures of 200 fish, where the true contribution of each is 1.0 under conditions of original baseline sample sizes (N).

most positively on PC2 were "stockier" with shorter and shallower heads, bodies, and caudal regions relative to the more elongated shapes of fish that had high negative scores along PC2 (Supplemental Fig. S2[2]).

The jackknifed DFA resulted in 75.9% correct morphological assignment, with two-thirds of the miss-assignments occurring owing to fish from morphological group 1 (MG1) assigned to group 2 (MG2). There was slight, but significant, association between geography and morphological group; MG1 predominated in the south arm of Atlin Lake (P = 0.046; Fig. 4). By contrast, there was no significant association (P = 0.35) between morphotype composition and the three STRUCTURE-generated genetic subpopulations identified earlier.

The brightness scores ranged from a low of near 100 (representing dark shading with distinct white spotting) to over 120 (representing silver–white shading with indistinct light spotting; Fig. 5). Average brightness scores were not statistically different among the three Atlin Lake genetic subpopulations (P = 0.13) or the two morphological clusters (P = 0.15). The observed geographical distribution of brightness scores was, however, found to be significant (P = 0.016), with the higher mean scores (lighter variety) being more common in the south arm (Fig. 5). An F_{ST} of 0.004 (95% CI of –0.001 to 0.009) was calculated between the two morphological clusters, six times lower than the value of the global F_{ST} of 0.022 among the three genetic subpopulations. Finally, analysis using STRUCTURE provided no evidence of more than one genetic population within the group of fish examined morphologically; log-likelihood was highest at $K(1)$ = –2731, with $K(2)$ having the next highest likelihood at –2939.

Discussion

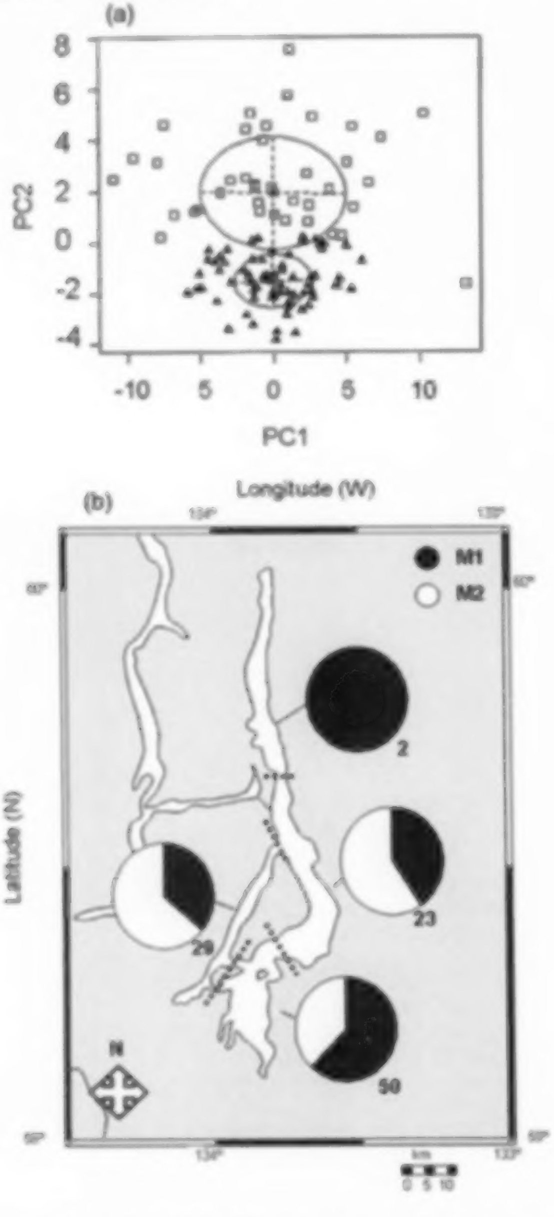
The ability to detect genetic substructure within populations has greatly increased over the last decade, which can provide information to promote effective management (Schwartz et al. 2007). Our study examined the level of genetic diversity and subdivision of lake trout within Atlin Lake and other western lake trout populations to help facilitate comparisons with other populations in more heavily studied and perturbed systems of eastern North America. These data can be used as baseline levels of genetic diversity for more effective conservation and management decisions for lake trout both in Atlin Lake and more generally.

Genetic variability across lakes and regions

Our study yielded significant F_{ST} values that ranged from 0.014 between two lakes within the same watershed and connected by a short river (Atlin and Tagish lakes) to 0.616 between two lakes (Trapper Lake, Taku River watershed, and Bednesti Lake, Fraser River watershed) that are separated by about 780 km, located in different watersheds, and are thought to have been colonized by fish from distinct glacial refugia (Wilson and Hebert 1998). The overall mean pairwise F_{ST} of 0.25 is well within the range reported among populations of freshwater resident salmonid fishes that, as a group, tend to have higher levels of genetic subdivision than anadromous salmonids (Hendry et al. 2004). Substantial subdivision among freshwater populations of salmonids, despite the excellent dispersal potential of salmonid fishes, is consistent with the generally greater constraints on interlocality dispersal imposed by the underlying landscape in freshwater environments.

Where opportunities for dispersal do exist such as be-

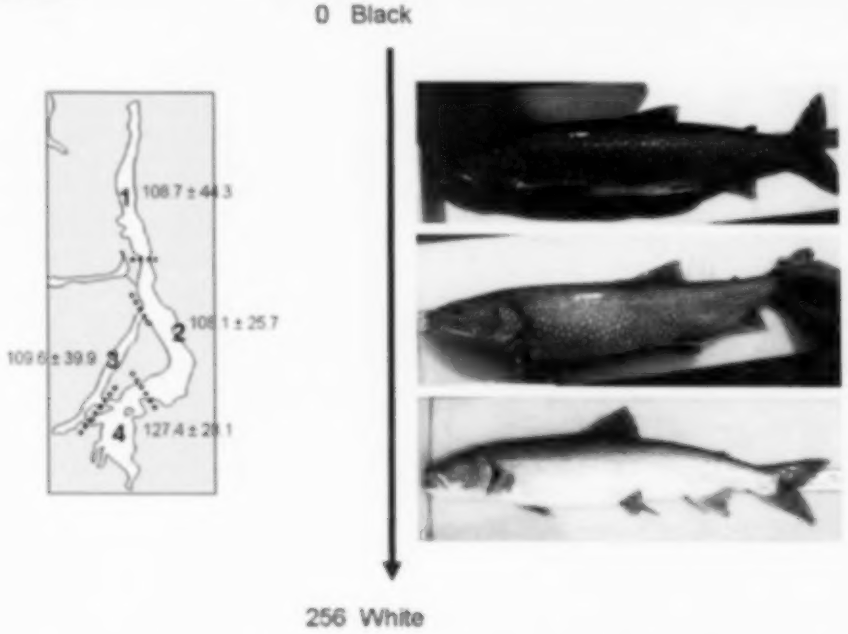
Fig. 4. (*a*) Morphological variation among Atlin Lake lake trout (*Salvelinus namaycush*) along the first and second principal components (PC1 and PC2). Ellipses represent the covariances of the values along each component, solid triangles correspond to morphological group 1 (MG1), and open squares correspond to morphological group 2 (MG2). (*b*) Map of the distribution of the two morphological groups among four geographic regions within Atlin Lake. The value to the right of each pie chart is the sample size for each region of the lake.



tween Atlin and Tagish lakes, genetic subdivision is lower. More modest levels of subdivision (expressed as G_{ST} and F_{ST}) of between 0.024 and 0.058 were reported among historical and contemporary wild and hatchery populations of lake trout within Laurentian Great Lakes Huron and Superior (Guinand et al. 2003; Page et al. 2004). Measures of subdivision are expected to be lower when more continuously distributed populations (e.g., within lakes Huron and Superior) are compared relative to widely separated or completely isolated populations (e.g., our study lakes, among Lakes Superior and Huron, and upper Mississippi River drainage lakes; DeWoody and Avise 2000; Page et al. 2004; Piller et al. 2005). Consequently, the large spatial scale of our study (Northwest Territories to central BC) probably accounts in large part for the strong differentiation that we observed. These results reinforce the importance of geographic proximity as an important factor influencing genetic structure among populations (Slatkin 1993; reviewed by Bohonak 1999). It is, however, more surprising that a signature of isolation-by-distance was detected among lake-dwelling populations within the Fraser River basin because these populations are strongly isolated currently. Consequently, there is almost surely very little contemporary movement between these lakes (i.e., although lake trout may occur in rivers and occasionally spawn there, they are generally associated with deepwater, lacustrine habitats; Scott and Crossman 1998). These results suggest that historical patterns of recolonization (timing, direction, sequence of events) may play some role in influencing current patterns of genetic structure even among relatively isolated populations (Crispo and Hendry 2005).

Analyses based on morphology and mtDNA have suggested that the Atlin–Tagish lakes area was colonized postglacially by fish from two refugia: the Nahanni Refuge and the Bering Refuge. By contrast, the lower Mackenzie River (e.g., Travaillant Lake) and the Fraser River watersheds are thought to have been colonized by fish from the Bering Refuge only (Bodaly and Lindsey 1977; Wilson and Hebert 1998). Our results are consistent with these hypotheses given that the Atlin–Tagish samples were very distinct from the Travaillant Lake and Fraser River samples, suggesting that they have had distinct origins postglacially. Further, our microsatellite data suggested that the Yukon and Taku rivers' watershed lakes are as similar to each other as are the lakes within the Fraser River watershed. The similarity between the Yukon (draining to the Bering Sea) and Taku (Pacific drainage) rivers is consistent with a common origin for these fish and (or) with suspected historical drainage connections between watersheds of these major river systems in the southern Yukon Territory (Lindsey 1975). By contrast, the sources of lake trout in the Mackenzie River watershed appear to be more variable. Travaillant Lake (lower Mackenzie River, Arctic drainage) lake trout were more similar to lake trout from the Fraser River watershed (Pacific drainage) than to fish from a lake tributary to the upper Mackenzie River (via the upper Liard River, Arctic drainage) — Frances Lake. Fish from Frances Lake, in turn, were more similar to lake trout from the Yukon and Taku rivers. The latter result is consistent with the shorter distance between Frances Lake and the upper Yukon River, even though Frances Lake is part of the upper Liard River, which ultimately flows to the Mackenzie River via the Peace River. In addition, Frances Lake lies just west of the area thought to have encompassed the Nahanni Refuge, which probably provided some postglacial colonists to the southwestern Yukon

Fig. 5. Results of comparisons of average (± standard deviation, SD) brightness values for lake trout (*Salvelinus namaycush*) sampled from the four geographic regions within Atlin Lake. Sample sizes are given in Fig. 2, and images illustrate the extremes of colouration of lake trout found within Atlin Lake.



(Wilson and Hebert 1998; Stamford and Taylor 2004). These patterns highlight the importance of considering historical patterns of connectivity in the interpretation of genetic affinities among contemporary populations in lake trout, a phenomenon shared with other fishes from geologically active areas (Currens et al. 1990; Waters and Wallis 2000; Zemlak et al. 2008).

Genetic variability within and between Atlin and Tagish lakes

Atlin and Tagish lakes were the only two populations that displayed deviations from HWE across multiple loci, which suggested that some substructure might be present, an inference supported by the STRUCTURE analysis. Genetic subdivision within single geographic localities is common in salmonids (summarized by Hendry et al. 2004), including lake trout (Ihssen et al. 1988; Page et al. 2003), and might have been expected in Atlin and Tagish lakes as they are by far the largest lakes in our survey. The level of subdivision found among the three genetic subpopulations in Atlin and Tagish lakes (F_{ST} = 0.022) was greater than between Atlin and Tagish lakes when the fish were simply grouped by lake and not accounting for genetic substructure within lake (F_{ST} = 0.014). Atlin Lake and Tagish Lake are connected by the Atlin River where lake trout do occur (M. Connor, Taku River Tlingit First Nation Fisheries Department, Atlin, BC, unpublished data). Interlake dispersal via this river probably explains the finding of the same genetic groups between the lakes and, therefore, that fish found in either Atlin Lake or Tagish Lake may be more similar to fish found in the other lake. In addition, the generally higher levels of subdivision observed in Tagish Lake relative to Atlin Lake may result from our sampling of actual spawning reefs in Tagish Lake, sampling that should yield a better estimate of genetic structure as a function of reproductive isolation than the more general lake survey sampling of nonreproductive adults completed in Atlin Lake.

Subpopulations within Atlin and Tagish lakes appeared to exhibit comparable levels of subdivision (F_{ST} = 0.005–0.034) with those reported among populations separated over similar distances within individual Laurentian Great Lakes (Ihssen et al. 1988; Page et al. 2004). By contrast, these values are somewhat lower than the maximum values reported from a study of spawning reefs within Lakes Huron, Michigan, and Superior (F_{ST} = >0.1) (Page et al. 2003), but these latter samples represented spawning populations that had been repopulated after stocking of fish from diverse hatchery sources.

Biochemical genetic and tagging data both strongly suggest that some lake trout exhibit very precise homing, returning to their spawning site year after year (Ihssen et al. 1988). These studies also indicated that the typical dispersal range of most lake trout is about 30 km but can be upwards of 50 km, but usually no more than 100 km, from their spawning shoals (Ihssen et al. 1988). Lake trout populations may also utilize environmental cues to select spawning beds, suggesting that distance is not the sole isolating factor (Perkins et al. 1995). In Atlin Lake, the spawning shoals identified thus far are all within the central portion of the lake. Consequently, the location of these spawning beds in close proximity to each other may be one of the reasons for such apparent high gene flow in Atlin Lake, but large areas of the lake remain unsurveyed for spawning areas. In Tagish Lake, three spawning bed locations had sufficiently high sample numbers to determine if they were primarily from one genetic subpopulation or not. Genetic subpopulation ATL-C dominated the spawning bed furthest west, while the spawning beds in the eastern and southern portions of the lake were characterized by higher proportions of subpopulation TAG-B, suggesting some independence among spawning areas.

Phenotypic vs. genetic diversity

Local residents have reported multiple colour and shape morphs of lake trout in Atlin Lake for generations, but whether the different morphotypes were discrete populations or represented a continuous range of variation was not known (M. Connor, Taku River Tlingit First Nation Fisheries Department, Atlin, BC, unpublished data). Our study suggests the presence of two morphological kinds of lake trout within Altin Lake that appear to differ primarily in head shape, body depth, and fin position. The three morphotypes found within the Laurentian Great Lakes exhibit significant differences in head shape (Moore and Bronte 2001) and the Great Bear Lake "redfin" and "normal" morphotypes differ in head size and dorsal fin position (Alfonso 2004). Independent from these morphotype differences was variation in brightness in fish from across Atlin Lake. Zimmerman et al. (2006, 2007), in contrast, found brightness differences to be associated with different body forms and that they tended to be collected from different water depths. Similarly, geographic segregation of colour morphs of lake trout has also been reported in Great Bear Lake (i.e., light in the south, dark in the north), but the basis of this pattern is unknown (Blackie et al. 2003).

The striking difference in brightness of fish observed in Atlin Lake is most likely promoted by variability in the lake's environment, especially in terms of turbidity and possibly water colour. The high level of turbidity caused by the influx of sediment from glacial meltwater characterizes the lake's south arm where the distinctive bright "silver" shading of the lake trout predominates. In fact, locally, these fish are known as the "glacial" variety. Colouration differences found in Lake Mistassini (Zimmerman et al. 2007) showed individuals in the deeper waters to be lighter-shaded than those in shallow water. Although all fish captured in the Atlin Lake survey were found in depths of less than 50 m, the increased turbidity caused by the glacial meltwater in the south end of the lake may be simulating the conditions in deeper waters. By contrast, lake trout in Great Slave Lake (Zimmerman et al. 2006) exhibited the reverse pattern (i.e., light in shallow water, and dark in deep water). Perhaps if fish in Atlin Lake were caught in a greater range of water depths, an association between depth and shading–colour–brightness might also be found. The extent to which such brightness variants are the result of genetic differences or result from an environmentally induced phenotypic response is, however, unknown (Miller et al. 2007; Stuart-Fox and Moussalli 2009). Indeed, there is a long history of morphological investigation in salmonids, and such variation can be primarily environmental, genetic, or both in origin (e.g., Pakkasmaa and Piironen 2001; Imre et al. 2002; Keeley et al. 2007). The three main morphotypes of lake trout found in some portions of the Laurentian Great Lakes (so-called "humper", "siscowet", and "lean" varieties) explained a greater percentage of total microsatellite variation than locality, suggesting that morphological groups may also represent distinct genetic groups in lake trout (Page et al. 2004; Bronte and Moore 2007). Furthermore, lake trout raised in hatcheries maintain morphological differences among parental groups, suggesting that a genetic component is involved (Moore and Bronte 2001). As yet, however, no genetic information has been provided for the other morphologically diverse lake trout in the Mackenzie Great Lakes. Whether that would be the case of Atlin Lake lake trout and whether or not such differences are associated with fitness are uncertain as the necessary common garden, performance, or selection experiments have not been performed (Grewe et al. 1994; Perkins et al. 1995; Bronte and Moore 2007).

Despite the identification of two morphotypes (independent of brightness differences), there was considerable overlap between the types, which was confirmed by a level of miss-classification of approximately 26% by discriminant function analysis. Because of the overlap and subtle differences between the morphotypes, it would be difficult to assign an individual fish to one type or the other in the field by visual identification. This presence of different morphotypes in Atlin Lake could be driven, in part, by sexual dimorphism, and we were unable to discount this possibility by sexing all of our fish. Sexual dimorphism in lake trout, however, is much less distinct than in other char, even when spawning (McPhail 2007), and there was no obvious way for us to sex our prespawning samples externally. Also, Zimmerman et al. (2007) found no association between sex and either morphological or brightness variation in Lake Mistassini lake trout. In addition, in the few mortalities that did occur during our sampling, sex appeared to be randomly distributed between morphotypes (two females and one male were in MG1 and one male and one female were in MG2). The brightness differences are unlikely to be explained solely by sexual dimorphism given the clear association of brightness scores with lake water colour (clear vs. glacial flour colour in the south arm) and associated crypsis via background matching (sensu Stevens and Merilaita 2009). Finally, because the morphotypes–brightness differences showed significant geographic associations in Atlin Lake, if such variation was driven by sexual dimorphism, one would need to invoke sexual differences in habitat use to explain the association between phenotype and geography.

The morphological subpopulations resolved in Atlin Lake did not correspond to the subpopulations defined through microsatellite analysis. This could result from environmental differences among localities that control the expression of the phenotypes among members of different genetic subpopulations that use a range of environments during nonspawning periods. Alternatively, there could be considerable gene flow at neutral microsatellite loci among lake trout subgroups characterized by adaptive differences in morphology, and there are many theoretical and empirical grounds for expecting adaptive divergence in phenotype in the presence of some gene flow at neutral loci (e.g., McKay and Latta 2002; Saint-Laurent et al. 2003; Hendry and Taylor 2004).

Our data are too few for a definitive analysis of any association between the established morphotypes from the Laurentian Great Lakes and those found in Atlin Lake. Casual visual identifications based on the morphological descriptions suggest, however, that Atlin Lake contains both a lean (streamlined shape and generally inhabiting water < 70 m deep) and a siscowet-like variety (deeper-bodied inhabitants of waters 70–150 in depth; Page et al. 2004). Unfortunately, none of the siscowet type could be included in our morphological analysis because of poor quality photographs. A more comprehensive comparative analysis across lakes could determine if the recognized morphotypes such as the siscowet reported from the Laurentian and Mackenzie Great Lakes are morphologically similar to those in Atlin Lake and if such similarity results from parallel evolution. Regardless of their exact identity or affinities, Atlin Lake is relatively small compared with other lakes where multiple morphotypes and genetic populations have been reported. This morphological diversity may be promoted by the physical connectivity between Atlin and Tagish lakes and the increasing habitat complexity that this represents.

Fisheries contributions and conservation

It is not surprising that some percentage (~22% on average) of the samples collected from Atlin Lake recreational and commercial fisheries was estimated to constitute fish from Tagish Lake owing to known incidences of dispersal of lake trout through the Atlin River, which connects Atlin and Tagish lakes. Lake trout caught by angling have a lower proportional contribution from Tagish Lake than do those from the commercial fisheries. This seems reasonable because the Atlin River is closer to the commercial fisheries locations than areas that receive the greatest angling effort.

Genetic mixture analysis estimated that genetic subpopulation ATL-C was most frequently sampled by both commercial operators and anglers. This subpopulation also possessed the largest estimated effective population size, which might explain its high estimated greater contribution to fisheries if this reflects a higher census size (Northrup 2008). In fact, baseline fish assigned to ATL-C were the most or second most abundant of all fish sampled in both Atlin (60%) and Tagish (42%) lakes. The contribution of ATL-C to fisheries in Tagish Lake is unknown due to the lack of samples from fisheries in Tagish Lake. In addition, given its high relative frequency, ATL-B probably contributes substantially to the Tagish Lake fisheries. Proximity of fishery areas to main feeding or reproductive habitats of subpopulations may contribute to unequal fishery contribution. In fact, the known spawning locations in Atlin Lake are found in its central portion and this area is the most heavily used by anglers. Therefore, if the genetic subpopulations are driven largely by homing to specific spawning beds, then those subpopulations closer to popular angling locations could show a higher contribution. In terms of regions within lakes, however, our mixture estimates had broad confidence intervals and thus interpretations of differential exploitation of the fish sampled from particular regions of either lake must be interpreted cautiously. Our simulated mixture results suggested that sufficient microsatellite variability was assayed across the eight loci to generate resolution consistent with previous studies of mixture analyses of lake trout, at least at the level of lake or genetic subpopulation (Page et al. 2003; DeKoning et al. 2006). Our simulations, however, also indicated that increasing baseline sample sizes would improve confidence for mixture analyses at smaller spatial scales within lakes.

Our study has resolved microsatellite allele frequency differences between samples across a broad geographic scale in British Columbia, Yukon, and the Northwest Territories and within single lakes of the upper Yukon River watershed. We also found evidence of morphological and colouration differences between localities within Atlin Lake, BC, indicating that such phenomena are general ones for lake trout across their geographic range. It is well known that the extent of genetic substructure within a population assemblage linked by gene flow and its influence on effective population size can have important effects on the time to fixation or loss of alleles, as well as which alleles are more likely to fix (e.g., Whitlock and Barton 1997; Whitlock 2003). Following these principles, the lake trout substructure of Atlin and Tagish lakes is an important attribute that can impact the rate of evolution of lake trout and hence influence their persistence in the face of environmental change (Whitlock 2003). Consequently, to sustain lake trout and their fishery potential within the large and complex ecosystem of Atlin–Tagish lakes, managers should consider and maintain the morphological and molecular biocomplexity that we have documented and the interaction of subpopulations within and between these two lakes (cf. Hilborn et al. 2003). The evolutionary potential of lake trout, and ultimately their persistence, in this large lake ecosystem and others like it most likely depends on the survival of many subpopulations across a diversity of habitats within such large lake basins. More broadly, previous conceptions of lake trout ecology such as a narrow ecological niche and limited tolerance to temperature and oxygen levels, based largely on the Laurentian Great Lakes populations, have been revised based on new information (Wilson and Mandrak 2003). If the Laurentian Great Lakes are quantitatively different, as some have suggested (Wilson and Mandrak 2003), then obtaining knowledge of populations outside this area such as we have provided offers a more complete understanding of the biology of lake trout.

Acknowledgements

Funding and field assistance for this project was provided by the Taku River Tlingit Fisheries Department and the Yukon Territory Government and an Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant awarded to E.B.T. We appreciate the assistance of Sean Rogers, Dolph Schluter, Patrick Tamkee, Jen Gow, and Luke Harmon with the various analyses. We also thank all of the people who assisted in collecting and providing samples and three reviewers for helpful comments on the study.

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Effects of temperature and elemental concentration on the chemical composition of juvenile yellow perch (*Perca flavescens*) otoliths

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Abstract: Otolith microchemistry studies assume that a relationship exists between the concentration of trace elements in the environment and otolith chemical composition. Although this assumption has been tested using marine and estuarine fish in controlled laboratory experiments, the relationships among temperature, ambient elemental concentration, and otolith chemical composition for freshwater species is not well documented. Here, juvenile yellow perch (*Perca flavescens*) were reared under different concentrations of four elements (Ba, Mg, Mn, and Sr) crossed with three temperatures (10 °C, 15 °C, and 20 °C) to determine the interactive influence of ambient elemental concentrations and temperature on otolith chemical composition. Sr:Ca and Ba:Ca were significantly related to ambient elemental concentrations, but Mg:Ca and Mn:Ca were not. Although the relative influence of temperature was less than that of ambient elemental concentrations, Sr:Ca, Ba:Ca, and Mn:Ca were all influenced by either water temperature or the interaction between temperature and elemental concentration, but the direction of the temperature effect differed for each element. Patterns in our partition coefficients are consistent with the idea that uptake of strontium facilitates uptake of barium. Overall, yellow perch otolith element composition was influenced primarily by ambient Sr and Ba concentrations, but temperature could potentially confound the results of otolith microchemistry studies.

Résumé : Les études microchimiques des otolithes présupposent l'existence d'une relation entre la concentration des éléments en trace dans l'environnement et la composition chimique des otolithes. Bien que cette présupposition ait été vérifiée chez des poissons marins et estuariens dans des expériences contrôlées en laboratoire, la relation entre la température, les concentrations ambiantes d'éléments et la composition chimique des otolithes chez les espèces d'eau douce n'a pas été bien établie. Nous avons élevé ici de jeunes perchaudes (*Perca flavescens*) dans diverses concentrations de quatre éléments (Ba, Mg, Mn et Sr) combinées à trois températures (10 °C, 15 °C et 20 °C) afin de déterminer l'influence interactive des concentrations ambiantes d'éléments et de la température sur la composition chimique des otolithes. Sr:Ca et Ba:Ca sont en relation significative avec les concentrations ambiantes d'éléments, mais Mg:Ca et Mn:Ca ne le sont pas. Bien que l'influence relative de la température soit moindre que celle des concentrations ambiantes d'éléments, Sr:Ca, Ba:Ca et Mn:Ca sont tous influencés ou bien par la température de l'eau ou alors par l'interaction entre la température et la concentration d'éléments, mais le sens de l'effet de la température diffère pour chacun des éléments. Les patrons dans les coefficients de partition obtenus sont compatibles avec la notion que l'incorporation de strontium facilite l'incorporation de barium. De façon globale, la composition des éléments dans les otolithes de perchaudes est influencée principalement par les concentrations ambiantes de Sr et Ba, mais la température pourrait potentiellement fausser les résultats d'études sur la microchimie des otolithes.

[Traduit par la Rédaction]

Introduction

Biogeochemical techniques have been used to study a broad range of ecological questions using many different types of animals (Rubenstein and Hobson 2004). Some examples include identification of overwintering habitat of Monarch butterflies (*Danaus plexippus*) (Wassenaar and Hobson 1998), essential foraging habitat for loggerhead turtles (*Caretta caretta*) (Hatase et al. 2002), long-distance migration patterns of baleen whales (Hobson et al. 1999), and habitat use and diet of bird populations (Bearhop et al. 2003). Fishery ecologists, in particular, have used biogeochemical markers to answer questions about the life history of fish. Specifically, biogeochemical makers have been used to identify the natal origins of adults (Atlantic salmon, *Salmo*

Received 22 May 2009. Accepted 19 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 25 June 2010.
J21214

Paper handled by Associate Editor Bronwyn Gillanders.

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Can. J. Fish. Aquat. Sci. **67**: 1187–1196 (2010)   doi:10.1139/F10-050   Published by NRC Research Press

salar; Kennedy et al. 1997), spatial structuring of spawning populations and homing behaviors (weakfish, *Cynoscion regalis*; Thorrold et al. 2001), and movement patterns (westslope cutthroat trout, *Oncorhyncus clarkii lewisi*; Wells et al. 2003) in fish.

By far, the most common biogeochemical methods used in fish ecology involve measuring the concentration of trace elements in otoliths. Otoliths, or earstones, grow continuously throughout the lifetime of the fish, accreting daily rings that are similar to the layers of an onion. As the otolith grows, trace elements are incorporated from the water into the otolith. Otoliths are metabolically inert; therefore, the trace elements incorporated into the otolith are not resorbed and represent a permanent record of the environmental history of the fish (Campana and Neilson 1985). Trace elements typically studied in fish otoliths include Sr, Ba, Mg, Li, Pb, and Mn, with the first two elements being the primary focus. The process of elemental uptake is complex and element-specific (Elsdon and Gillanders 2003); however, high elemental concentrations in water typically are correlated with high elemental concentrations in the otolith. Using laser ablation technology, it is possible to pinpoint a small area of the otolith that represents a precise age (e.g., the larval period) and then associate the elemental composition of the otolith with that specific age.

This technique is increasingly being used in studies of life history and movement patterns in freshwater fish. In many of these studies, investigators sample fish at one life stage and use otolith analysis to determine where the fish were at an earlier life stage, without having samples of fish directly from that earlier life stage. In these cases, investigators must rely on being able to relate elemental concentrations sampled from the part of the otolith formed during the early life stage with spatial differences in elemental concentration in the water among potential locations of fish during that life stage. There are currently some gaps in our understanding of the elemental uptake process in freshwater systems that limit our ability to complete such analyses. In a recent paper, Elsdon et al. (2008) reviewed five methods for determining fish movement patterns based on the chemical composition of otoliths and the assumptions implicit with each method. Of the five methods reviewed, two methods in particular, profile analysis to define life history variation within an population and profile analysis to describe movements through different environments, make two key assumptions that, to date, have not been tested using fish that reside solely in freshwater habitats. These assumptions, that a predictable relationship exists between the elemental concentration of the water and the resultant elemental concentration in the otolith and that the interactive effect of environmental parameters on otolith chemistry are known (Elsdon et al. 2008), remain untested, despite the fact that researchers are increasingly using otolith chemistry to answer questions about movements of fish across habitats in freshwater ecosystems (Whitledge et al. 2007; Pangle et al. 2010; Whitledge 2009). Of all of the potential environmental factors that may influence otolith elemental uptake rates, the relationships between temperature and otolith chemistry have been most widely examined in laboratory studies of marine and estuarine fishes (Fowler et al. 1995; Bath et al. 2000; Martin et al. 2004). Although it is likely that temperature will influence chemical uptake in the otoliths of freshwater species, its exact influence has yet to be established. Because environmental concentrations of elements are lower in freshwater systems than in marine systems and because the physiological mechanism of elemental uptake differs substantially between marine and freshwater fish (Olsson et al. 1998), we cannot rely entirely on previously established findings from marine studies (Wells et al. 2003; Hedges et al. 2004). Characterizing how the environmental concentration of trace elements and temperature influence uptake rates by freshwater fish is necessary if this technique is to be applied successfully in freshwater environments.

Here we describe the results from a series of experiments that were conducted to characterize the elemental uptake process in juvenile yellow perch otoliths. Spatial variation in yellow perch otolith elemental signatures has been documented in field studies (Brazner et al. 2004*a*, 2004*b*), but laboratory validation of key assumptions regarding otolith chemistry is lacking for this primarily freshwater species. These experiments, conducted during the summers of 2005 and 2006, were designed specifically to quantify the interactive effects of ambient elemental concentrations and temperature on otolith chemistry. By measuring the interaction between elemental concentration and temperature on the elemental concentration in otoliths, we should improve the accuracy of otolith chemistry studies in freshwater systems. Our ultimate goal is to use the relationships generated from these experiments to improve the interpretation of field-based studies in Lake Erie, but we expect our results to be useful for other freshwater studies, as well. Because Lake Erie spawning and larval-rearing habitats experience a range of temperatures across locations at a given time and within locations over a season, we expect the problem of temperature to be important in these studies.

Materials and methods

During the spring and summer of 2005 and 2006, we conducted experiments to determine the influence of water elemental concentration and temperature on elemental uptake in yellow perch otoliths. Juvenile yellow perch were obtained from St. Mary's State Fish Hatchery, St. Mary's, Ohio, in May 2005 and 2006. Fish were hatched and reared at the hatchery using water from a common source prior to the experiment. For the experiment, fish were transported to the wet lab facilities of the Aquatic Ecology Laboratory (AEL) at The Ohio State University (Columbus, Ohio). Upon arrival at the AEL, all fish were acclimated to laboratory conditions for approximately two weeks while being held in a single recirculating system comprised of two 890 L circular tanks connected to a single biofilter.

Experimental design

Temperature and elemental concentration combinations represented a range of expected conditions in Lake Erie and its tributaries during the spring and early summer. Temperature treatments were maintained at 10, 15, and 20 °C. Elemental concentrations consisted of nonspiked laboratory water, which served as a baseline, and dechlorinated tap water spiked with low and high concentrations of strontium, barium, magnesium, and manganese. Elemental levels were established by adding appropriate amounts of trace metal grade $SrCl_2$ (300 and 1500 $\mu g \cdot L^{-1}$ added to low and high elemental concentration treatments, respectively), $BaCl_2$ (35 and 70 $mg \cdot L^{-1}$), $MgCl_2 \cdot 6H_2O$ (20 and 40 $mg \cdot L^{-1}$), and $MnCl_2 \cdot 4H_2O$ (10 and 40 $\mu g \cdot L^{-1}$) to dechlorinated water (Bath et al. 2000). Water was changed three times per week at a 50% volume to maintain water quality and elemental concentrations within the tanks. Throughout the experiment, fish were maintained on a 12 h light – 12 h dark cycle and fed ad libitum a diet of freeze-dried zooplankton.

Because of a shortage of fish in 2005, we ran two experiments, one during spring 2005 and another during spring 2006. In 2005, we conducted an experiment comparing baseline and high elemental-addition concentrations within three temperatures, with two replicate tanks for each treatment (Appendix A, Table A1). In 2006, an experiment comparing baseline and low elemental-addition concentrations within three temperatures was conducted, with one tank for each treatment (Appendix A, Table A1). At the onset of each experiment, juvenile yellow perch were randomly assigned to a 100 L tank. Fish were acclimated in experimental tanks for at least one week. During the acclimation period, temperature treatments were established by adjusting the environmental chambers to 10, 15, and 20 °C at a rate of 1 °C per day. After acclimation, fish were maintained in experimental treatments for 45 days. Water temperature and dissolved oxygen were monitored daily, and pH, ammonia, and nitrate levels were monitored every three days.

Elemental concentrations within the tanks were monitored by collecting water samples three times per week. Water samples were filtered through 0.45 µm cellulose nitrate membrane filters and preserved and acidified with 2% (by volume) trace metal grade nitric acid prior to elemental analyses. On day 45 of the experiment, all fish were removed from the tanks, euthanized with an overdose of tricaine methanesulfonate (MS-222), measured for total length, and weighed. To avoid contamination, otoliths were processed according to methods described in Fowler et al. (1995). Otoliths were handled using only acid-washed, nonmetallic instruments. We used glass probes to remove sagittal otoliths from juvenile fish under a dissecting microscope. Otoliths were prepared for elemental analyses by mounting them in epoxy, transverse sectioning near the otolith primordium using an Isomet low-speed saw, and polishing using 3 µm Imperial lapping film to expose the primordium. Sectioned otoliths were first cleaned by sonicating them in Super-Q water in an ultrasonic water bath, triple rinsed in Super-Q water, and air-dried in a class-100 hood. Dry otolith sections were mounted on acid-washed microscope slides using double-sided tape and stored in acid-washed polypropylene vials prior to chemical analyses.

Chemical analyses

All elemental analyses were performed by the Trace Element Research Laboratory (TERL) located at The Ohio State University. The concentrations of Ca, Ba, Mg, Mn, and Sr in the water from experimental tanks were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima 4300 DV spectrometer (PerkinElmer Inc., Waltham, Massachusetts). The instrument was calibrated using a set of standard solutions made by appropriate dilution and mixing of single-element standards (CPI International, Santa Rosa, California). Elemental concentrations in otoliths were measured using laser ablation inductively coupled mass spectrometry (LA-ICPMS) taken directly from solid samples using a UP-193HE 193 nm excimer laser (New Wave Research, Electro Scientific Industries, Inc., Portland, Oregon) with two 9 × 9 arrays of lenses to provide a fully homogenized laser beam. Aerosol generated by the ablation process was transported to an Element 2 inductively coupled plasma sector field mass spectrometer (ThermoFinnigan, Thermo Fisher Scientific Inc., Waltham, Massachusetts) in He (0.8 $L \cdot min^{-1}$), which flowed through the ablation cell. The He flowing out of the cell was mixed with 1.0 $L \cdot min^{-1}$ Ar and connected to the injector of the ICP torch. To avoid spectral overlaps at the $m \cdot z^{-1}$ of the major isotopes of Ca and to avoid signals that might be too large, a minor isotope ($^{43}Ca^+$, 0.135%) was measured. For other elements, we measured concentrations of the primary isotopes: $^{24}Mg^+$, $^{55}Mn^+$, $^{88}Sr^+$, and $^{138}Ba^+$. All elements were measured based on four points in the center 10% of the mass spectral peak using medium resolution ($m \cdot \Delta m^{-1}$ = 4000) with AutoLock mass to minimize mass drift.

During the early stages of chemical analysis, we conducted experiments to determine (*i*) an optimal laser configuration for LA-ICPMS analyses and (*ii*) precisely what part of the otolith to ablate to obtain accurate measurements of the uptake of elements from the water into the otoliths. Initially, LA-ICPMS measurements were made across the otolith along the longest axis, using both a line scan mode and a series of spots spaced 75 µm apart. The line scan provides continuous measurement of elemental concentrations across the otolith, but we chose a series of spots to avoid loss of lateral spatial resolution due to the approximately 10 s washout time of the New Wave Research ablation cell.

From our early chemical analyses, we constructed two-dimensional maps showing the spatial configuration of Sr concentration in otoliths from yellow perch that were reared under experimental conditions. The maps were generated using a series of LA-ICPMS spot measurements arranged in a regular, grid-like pattern across the surface of the cross-sectioned otolith, and Sr concentrations across the exposed surface of the otoliths were interpolated using contour plotting in SigmaPlot 10 (Systat Software Inc., San Jose, California). From the otolith maps, it was clear that uptake of elements from the tank water occurred mainly in the outermost 50–75 µm of the otolith (Fig. 1). Based on maps produced from both baseline and experimental otoliths, we concluded that the best region to sample was the region on the ventral side of the otolith, below the sulcus. A 30 µm laser spot size was used to provide resolution so that the area of the otolith being sampled would provide a relatively homogeneous elemental signature while also providing sufficient material for detecting small concentrations of elements via ICPMS. The laser energy per area per pulse at the sample was approximately 15 $J \cdot cm^{-2}$. Data were acquired for each of the five isotopes, each second, for approximately 40 s at each ablation spot. A delay of 20 s was used between measurements at successive spots. All other isotope signals were ratioed to the $^{43}Ca^+$ signal to compensate for any changes in ablation rate over time or from sampling location to sampling location.

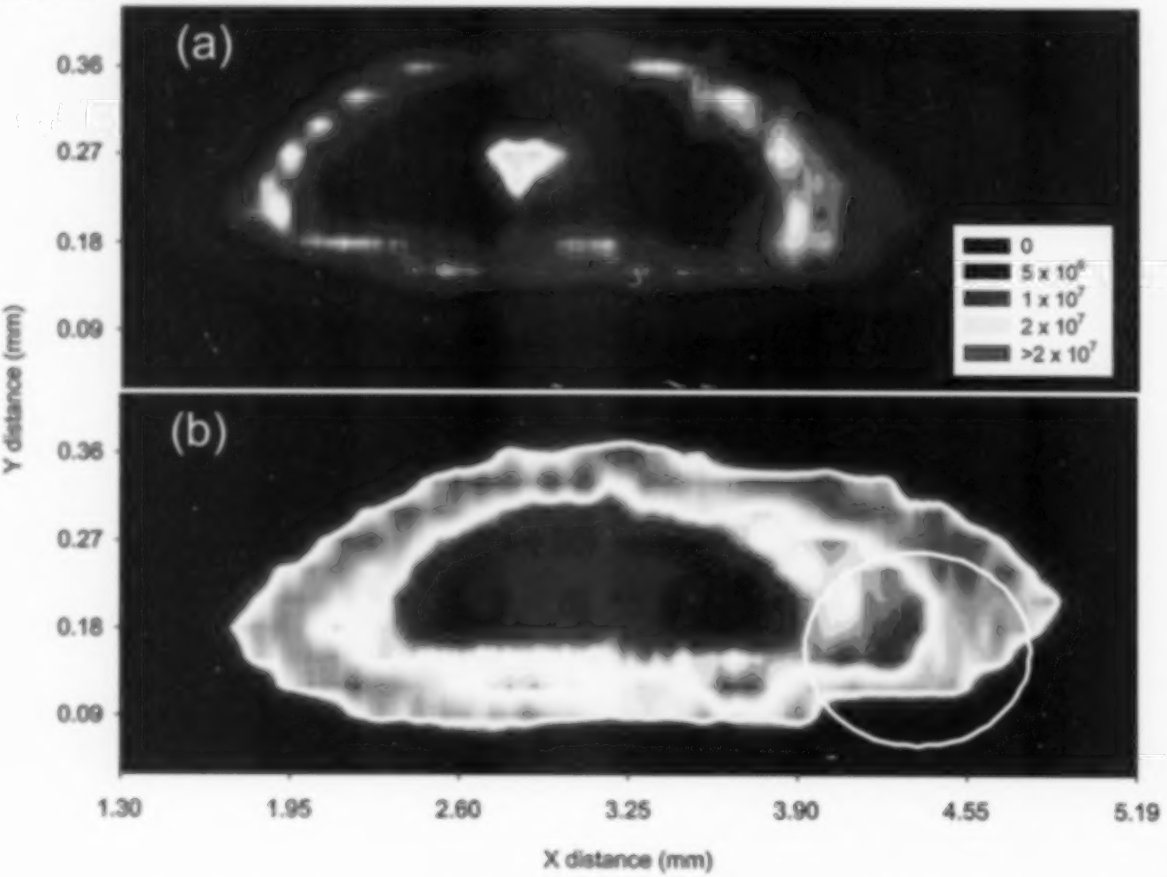


Fig. 1. Two-dimensional map of cross-sectioned juvenile yellow perch (*Perca flavescens*) otoliths demonstrating spatial differences in strontium uptake. Map (*a*) was drawn from measurements of an otolith from a fish reared in baseline elemental concentrations, and map (*b*) was drawn from measurements of an otolith from a fish that was reared in high elemental concentration water. Both fish were exposed to experimental conditions for 45 days at a temperature of 20 °C. Different colors on the map indicate inductively coupled plasma mass spectrometry (ICPMS) signal strength with respect to strontium, with cool colors (blue and green) indicating low strontium levels and hot colors (yellow and red) indicating high strontium levels. The white circle indicates the area of the otolith that was ablated using individual spots during subsequent laser ablation (LA) – ICPMS analyses.

Calibration was based on a blank measurement (i.e., shutter closed so that no laser radiation hit the sample) and a NIST 612 silicate glass standard reference material. Preferred concentrations were taken from published sources (Pearce et al. 1997). As a check for instrumental drift, NIST 612 was measured before and after each set of measurements of otoliths from fish in the treated and baseline water.

Data analysis

For all elements, the results from the ICPMS analysis were expressed as molar concentrations (mmol·mol^{-1} Ca) prior to statistical analyses. Natural isotopic abundances were assumed to be the same in the NIST 612 standard and the otoliths. For Sr and Ba, element-to-calcium ratios were calculated for each otolith based on laser pulses fired at five spots on the otolith. For some otoliths, visual inspection of the raw signal from the ICPMS revealed high background levels for our initial measurements of Mg and Mn. To correct for the background signal in the ICPMS, we subtracted background levels for all elements. In cases where initial background levels were high for Mg and Mn but decreased somewhat during the analysis, we used the subtracted background levels at the end of the analyses from the elemental signals prior to calculation of Mg:Ca and Mn:Ca. In these cases, only the last three spots measured on the otoliths were used because the background was stable before and after these spots. For each otolith, we calculated a coefficient of variation (CV) to determine the spot-to-spot precision for each element and, based on visual inspection of the CV distribution across all elements and treatments, excluded data that had CV values greater than 0.7. In addition, we calculated partition coefficients (D_{Me}, from Bath et al. 2000) for each element-to-calcium ratio,

$$\frac{[\mathrm{Me}_{\mathrm{otolith}}]}{[\mathrm{Ca}_{\mathrm{otolith}}]} = D_{\mathrm{Me}} \frac{[\mathrm{Me}_{\mathrm{water}}]}{[\mathrm{Ca}_{\mathrm{water}}]}$$

Table 1. Water elemental concentrations and element-to-calcium ratios (mmol·mol^{-1}) measured in the experimental tanks. Means of all days averaged over replicate tanks are reported with standard errors in parentheses. Elemental concentration treatments were compared using measurements of elemental ratios measured for each tank and date combination. Elemental ratios for all treatment levels were significantly different through the duration of the experiment (repeated-measures analysis of variance, $P < 0.05$). A range of elemental concentrations and elemental ratio values from Lake Erie are shown for comparison.

		Elemental concentrations (ppm)				Elemental ratios (mmol·mol^{-1})		
Treatment	Year	Ba	Ca	Mg	Sr	Ba:Ca	Mg:Ca	Sr:Ca
Experiment								
Baseline	2005	0.43 (0.01)	27.13 (0.08)	8.19 (0.05)	0.18 (0.01)	4.61 (0.06)	499.04 (3.33)	3.06 (0.16)
Baseline	2006	0.50 (0.01)	28.22 (0.24)	9.20 (0.08)	0.22 (0.02)	5.19 (0.10)	539.44 (4.53)	3.63 (0.37)
Low	2006	0.58 (0.01)	28.31 (0.26)	10.35 (0.08)	0.59 (0.01)	6.03 (0.14)	605.69 (2.94)	9.73 (0.09)
High	2005	0.58 (0.01)	27.01 (0.08)	10.91 (0.06)	0.94 (0.01)	6.32 (0.15)	667.65 (5.03)	15.87 (0.07)
Lake Erie								
Open lake[a]	2005	0.12–0.57	19.62–47.71	6.47–14.15	0.08–0.45	1.12–5.32	369.14–722.32	1.40–4.54
Tributaries[a]	2004	0.17–2.10	17.61–103.94	3.73–31.96	0.05–2.85	0.59– 12.67	146.24–775.63	1.10–16.26

[a] Data collected in the western and central basins of Lake Erie and western basin tributaries.

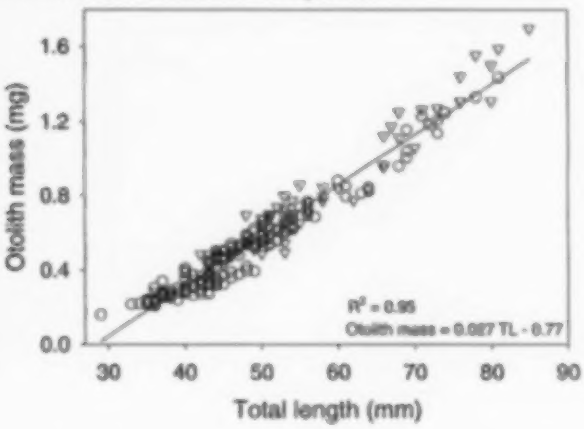


Fig. 2. Total length versus otolith mass for juvenile yellow perch (*Perca flavescens*). Fish from each elemental concentration in 10 °C (circles), 15 °C (triangles), and 20 °C (squares) temperature treatments were combined in the regression.

where [Me] represents the concentration of a given metal.

We used a repeated-measures analysis of variance (ANOVA) to compare the elemental composition of the water in the experimental tanks throughout the duration of the experiment. We used a nested ANOVA to test for the effect of water elemental concentration, temperature, and their interaction on element-to-Ca ratios in the otoliths, while accounting for any potential tank effects. Tank nested within the concentration by temperature interaction was used as the error term in tests of the null hypotheses of no effect of water elemental concentration, temperature, and their interaction. All statistical tests were performed using SAS 9.13 software (SAS Institute Inc. 2005) at a significance level of 0.05.

Results

Experimental conditions

Elemental concentrations were maintained among experimental tanks at levels that reflect conditions within Lake Erie and its tributaries throughout both years of experiments (Table 1). Mean element-to-calcium ratios differed among baseline and low and high elemental treatments for Ba, Mg, and Sr through the duration of the experiment during both years (repeated measures ANOVA, $p < 0.01$). We were unable to accurately measure Mn:Ca in the water because concentrations were below the detection limits of our ICP-OES; therefore, Mn was excluded from the partition coefficient analysis.

Juvenile yellow perch grew considerably during the 45 days of the experiment. By the end of the experiment, yellow perch length was similar among elemental treatments within a temperature (2005, $F = 0.381$, df = 1, $P = 0.539$; 2006, $F = 0.547$, df = 1, $P = 0.461$) but differed among temperature treatments (2005, $F = 102.3$, df = 1, $P < 0.0001$; 2006, $F = 7.34$, df = 2, $P = 0.001$). Fish that were reared in warmer water were much larger than those reared at low temperatures. The differences between fish sizes at different temperatures are reflected in the positive relationship between otolith diameter and total length for juvenile yellow perch (Fig. 2).

Yellow perch otoliths

We measured element-to-calcium ratios from a total of 238 juvenile yellow perch otoliths during both years. Sample size within each treatment varied and was dependent on survival of juvenile yellow perch to day 45 of the experiment. The minimum number of otoliths within our treatments was 15 (baseline, 20 °C) and the maximum was 26 (low elemental concentration, 15 °C).

Each element showed a unique relationship between elemental concentration in the water, temperature, and elemental concentration in the otolith (Fig. 3). Neither Mg:Ca nor Mn:Ca in the otolith was influenced by water elemental concentrations (Mg:Ca, $F_{[2,9]} = 0.57$, $P = 0.586$; Mn:Ca, $F_{[2,9]} = 0.57$, $P = 0.585$), temperature (Mg:Ca, $F_{[2,9]} = 0.25$, $P = 0.7831$; Mn:Ca, $F_{[2,9]} = 0.66$, $P = 0.540$), or the interaction between elemental concentration and temperature (Mg:Ca, $F_{[4,9]} = 1.10$, $P = 0.411$; Mn:Ca, $F_{[4,9]} = 0.63$, $P = 0.653$). There was a tank effect ($F_{[9,221]} = 3.73$, $P = 0.0002$) on otolith Mn:Ca, but not on otolith Mg:Ca. Otolith Sr:Ca increased with increasing elemental concentration in the water ($F_{[2,9]} = 38.54$, $P < 0.0001$) and with temperature ($F_{[2,9]} = 5.10$, $P = 0.033$) in otoliths from fish reared in low and high Sr concentrations, but decreased with temperature in

Fig. 3. Mean element-to-calcium ratios for (*a*) Mg, (*b*) Mn, (*c*) Sr, and (*d*) Ba in otoliths taken from yellow perch (*Perca flavescens*) reared under baseline (shaded bars), low elemental addition (open bars), and high elemental addition (solid bars) treatments. Error bars are standard errors.

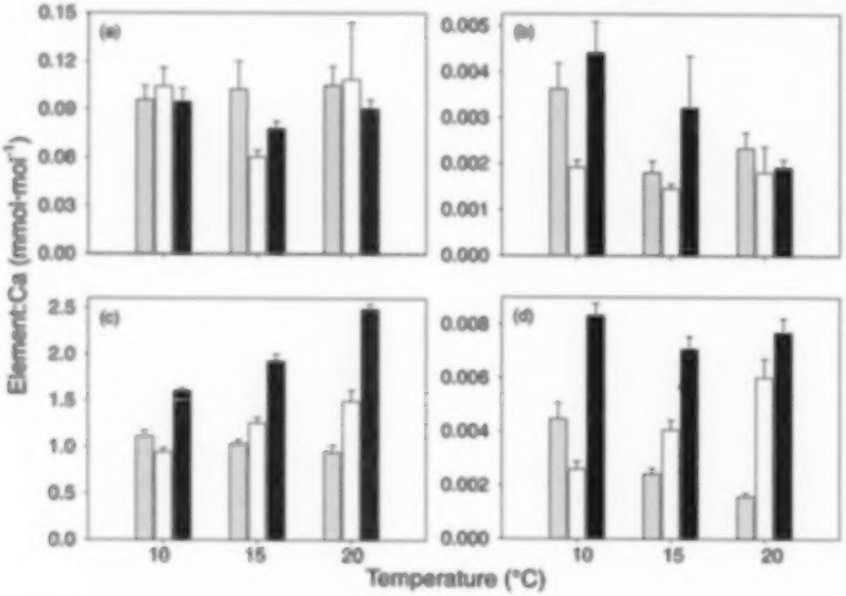
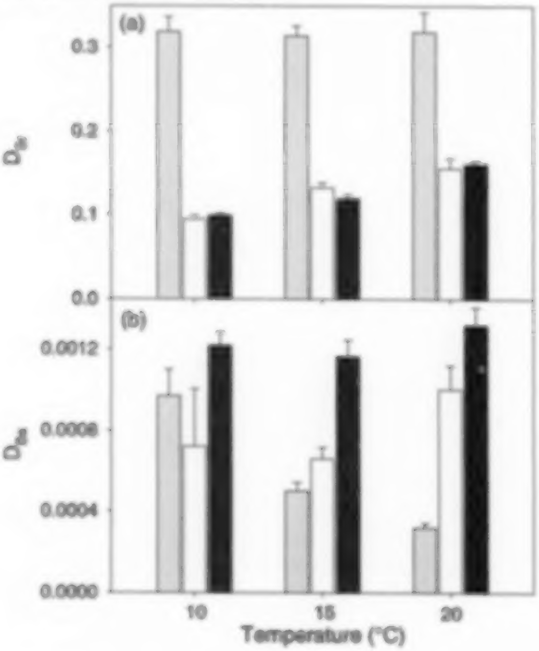


Fig. 4. Mean (*a*) strontium and (*b*) barium partition coefficients (D_{Sr} and D_{Ba}, respectively) for measured in yellow perch (*Perca flavescens*) reared under baseline (shaded bars), low elemental addition (open bars), and high elemental addition (solid bars) treatments. Error bars are standard errors.



otoliths from fish reared in baseline water (concentration × temperature interaction, $F_{[4,9]} = 5.08$, $P = 0.020$). Although there was a tank effect for otolith Sr:Ca ($F_{[9,221]} = 3.62$, $P = 0.0003$), the variance associated with tanks was minor compared with that associated with the main effects. Ba:Ca in otoliths increased as Ba concentration increased in the water ($F_{[2,9]} = 4.63$, $P = 0.0415$). Neither temperature ($F_{[2,9]} = 1.27$, $P = 0.326$), nor the interaction between ambient Ba concentration and temperature ($F_{[4,9]} = 0.18$, $P = 0.941$), nor tank had significant effects on otolith Ba:Ca ($F_{[9,221]} = 1.12$, $P = 0.349$).

As with element-to-calcium ratios, partition coefficients in yellow perch otoliths differed for individual elements (Fig. 4). Strontium partition coefficient D_{Sr} in yellow perch otoliths differed among levels of ambient Sr concentration ($F_{[2,9]} = 46.22$, $P < 0.0001$) but was not affected by temperature ($F_{[2,9]} = 1.08$, $P = 0.381$) or the interaction of ambient Sr concentration and temperature ($F_{[4,9]} = 0.40$, $P = 0.806$). A tank effect was present for D_{Sr} ($F_{[9,220]} = 4.87$, $P < 0.0001$), though the effect was small in comparison with the main effects. When compared with strontium, barium partition coefficients D_{Ba} were much smaller, with considerably more variation among different treatment groups. D_{Ba} was affected by ambient elemental concentration ($F_{[2,9]} = 4.3$, $P = 0.049$) but not by temperature ($F_{[2,9]} = 0.76$, $P = 0.494$) or the interaction between elemental concentration and temperature ($F_{[4,9]} = 1.03$, $P = 0.440$). A tank effect was present for D_{Ba} ($F_{[9,214]} = 5.35$, $P < 0.0001$).

Discussion

Many current applications of otolith microchemistry in studies of freshwater fish rely on the assumption that trace element concentration in otoliths is a predictable reflection of trace element concentration in the ambient water (Elsdon and Gillanders 2003). In the present study, we found that although element-to-calcium ratios in juvenile yellow perch otoliths were generally positively correlated with ambient elemental concentrations, the effect of water temperature produced results that could potentially confound conclusions in field-based studies.

Confounding environmental variables

The positive, predictable relationship between ambient Sr and Ba concentrations and otolith Sr:Ca and otolith Ba:Ca measured in the present study further demonstrates the utility of these elements for reconstructing the environmental history of freshwater fish. In the present study, Sr:Ca and Ba:Ca in juvenile yellow perch otoliths increased with increasing water elemental concentrations at most temperatures. The positive relationship between ambient elemental conditions and otolith Sr:Ca and Ba:Ca is consistent with results from numerous experiments in the marine and estuarine literature that have consistently linked ambient strontium and barium concentrations to otolith chemistry (Bath et al. 2000; Elsdon and Gillanders 2003; Kraus and Secor 2004). We also detected significant temperature effects on otolith Sr:Ca and Ba:Ca levels. If we ignore results from our coldest temperature (10 °C, see Elemental uptake at low temperatures), we found otolith Sr:Ca and Ba:Ca values to increase with increasing water temperatures in our two spiked treatments. Although we are unaware of any experiments that compare the relative effects of ambient elemental concentrations and temperature for freshwater fish, these results are consistent with previous studies from the marine literature that suggest that the rate of incorporation of elements, particularly strontium, into otoliths is influenced by water temperature (Radtke 1989; Fowler et al. 1995). In fact, the effects of water temperature on otolith elemental concentrations often exceed those of other environmental conditions such as salinity (Fowler et al. 1995). In spite of the temperature effects on Sr:Ca and Ba:Ca detected in our study, ambient elemental conditions are a better predictor of otolith chemistry than temperature, as depicted in the different relative magnitude of effects between ambient elemental effects and temperature effects for otolith Sr:Ca and Ba:Ca values.

Is the effect of temperature observed in our study significant enough to confound interpretation of patterns in field studies? We focus on Sr here, as we had a greater range in elemental concentration of Sr than of Ba. These results suggest that temperature could influence the results of otolith chemistry studies in field-caught fish because our experimental treatments were designed to mimic conditions that a yellow perch would experience in Lake Erie, with low Sr treatment approximating the level of environmental Sr in the Maumee Bay during early summer and the high Sr treatment approximating the level of Sr in Sandusky Bay during early summer, when water temperatures in Lake Erie reach 15–20 °C. If we assume a positive relationship between otolith Sr:Ca and ambient Sr:Ca concentrations as depicted in Fig. 3, we see that an otolith with Sr:Ca between 1.7 and 2.0 $\text{mmol}\cdot\text{mol}^{-1}$ could have come from 15 °C water having 15.9 $\text{mmol}\cdot\text{mol}^{-1}$ Sr:Ca or 20 °C water having 9.7 $\text{mmol}\cdot\text{mol}^{-1}$ Sr:Ca. If both of these combinations of temperature and Sr:Ca occur in the lake and major tributaries simultaneously, we would have some uncertainty in using otolith Sr:Ca alone to assign a location to this fish. Although Ba uptake also was affected by temperature at certain elemental concentrations, if the effect is different for Ba than for Sr, it may be possible to use information about both of these elements to interpret otolith elemental concentrations in field studies. If, for example, Sr and Ba concentrations increase in kind at a location, as they do with our experimental treatments, otolith Ba:Ca can be used to determine the origin of the fish because Ba:Ca in the otolith is not as heavily influenced by temperature as Sr:Ca.

The generality of results generated in our laboratory experiments ultimately depends on the consistency between laboratory-derived results and field-derived results (Thrush el al. 1997; Elsdon and Gillanders 2005). Unlike field conditions, where juvenile yellow perch would be exposed to varying environmental conditions, we attempted to hold most environmental conditions constant and measured only the effects of ambient elemental concentrations and temperature on otolith chemistry. After 45 days in experimental treatments, juvenile yellow perch otoliths showed strong differences in elemental concentrations across treatments, as well as within otoliths, in some cases. We chose to discard data when the CV within an otolith exceeded 0.7 for any element, which resulted in the exclusion of less than 5% of our data for certain elements. Although variation in the elemental composition within an otolith can be attributed to many factors that were unmeasured in the present study, we expect that the within-otolith variation that we recorded was likely the result of measurement error, and accordingly, these data were discarded. As a result, we expect that our results will be informative for a wide range of studies in systems with similar ranges of environmental conditions and for otoliths that are measured with a similar precision.

In addition to otolith elemental concentrations, we also calculated partition coefficients for Sr and Ba in yellow perch otoliths. In our study, strontium partition coefficients increased with water temperature, suggesting that Sr is incorporated into the otolith at a rate that is dependent on temperature. Although we are unaware of previous studies calculating partition coefficients for freshwater fish, the partition coefficients for Sr are comparable with those of previous studies using a variety of marine species, which estimated D_{Sr} at approximately 0.18 (Kalish 1991; Bath et al. 2000), compared with our estimate of 0.21. This suggests that in spite of physiological differences in elemental uptake mechanisms between marine and freshwater species and differences in the magnitude of ambient elemental conditions, uptake of Sr into otoliths from marine and freshwater fish occurs at a similar rate. In contrast, D_{Ba} was very low in our experimental tanks compared with published values for marine fish. This result could be attributed to the high ambient concentrations of Ba in our experimental tanks and in freshwater systems, in general. It has been suggested that at very high ambient Ba:Ca values, a threshold is reached and Ba can no longer incorporate into the lattice sites in the otolith $CaCO_3$ matrix (deVries et al. 2005; Miller 2009).

Trace elements such as barium and strontium are believed to be substituted into the otolith by substituting for calcium ions (Campana 1999); therefore, differences in uptake rate

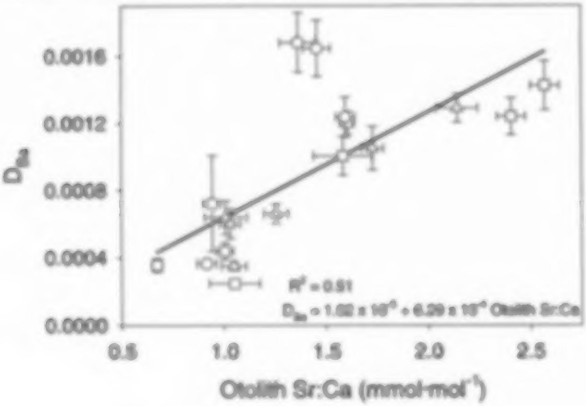


Fig. 5. Mean barium partition coefficients (D_{Ba}), calculated within each experimental tank, plotted against strontium-to-calcium ratios in otoliths from juvenile yellow perch (*Perca flavescens*) reared in tanks maintained at 10 °C (circles), 15 °C (triangles), and 20 °C (squares). A line was fitted using least-squares regression to demonstrate the facilitative relationship between strontium in the otolith and barium partitioning. Error bars are standard errors.

may occur due to competition for limited spaces in the otolith matrix. In the current study, we found that Sr is incorporated into the otolith more efficiently (higher D_{Sr} values) than Ba, suggesting different uptake rates for these elements. For juvenile yellow perch, strontium partition coefficients were higher for fish reared in baseline water than those reared in water spiked with strontium, suggesting that strontium is incorporated into otoliths more efficiently at lower ambient levels. On the other hand, barium partitioning was higher in otoliths from fish reared in both spiked treatments than from fish reared under baseline conditions at 15 °C and 20 °C. A study by deVries et al. (2005) found that rather than competing with strontium for space in the otolith, barium uptake into otoliths can be facilitated by high levels of strontium. Although we did not explicitly test whether high levels of Sr can influence Ba uptake rates, our results provide further support for the idea that high strontium concentrations can facilitate barium uptake into otoliths. From our data, we found a positive relationship between D_{Ba} and otolith strontium concentrations (Fig. 5), with otolith Sr:Ca explaining a considerable amount of the variance in D_{Ba}. In practical terms, these data illustrate the importance of measuring multiple elements from both water samples and otoliths because the uptake of one element may alter the relationship between otolith and ambient water conditions in other elements.

Contrary to our results with Sr and Ba, otolith Mg:Ca and Mn:Ca levels were not predictably influenced by ambient elemental concentrations or water temperatures. Although our results may seem to diminish the utility of these elements for otolith microchemistry studies in juvenile yellow perch, these elements may be useful in other applications. For example, very high levels of Mg and Mn have been detected in the core of otoliths from both freshwater and marine fish, indicating that these elements may be useful to address questions concerning the natal origins of larvae and larval exchange among populations in these systems (Ruttenberg et al. 2005). Moreover, the specific crystalline structure of otoliths may be an important indicator of the specific suite of elements that are incorporated into an otolith. For percids such as yellow perch, as well as most other teleosts, aragonite is the most common crystalline form of otoliths. However, vateritic otoliths have been detected in some species that reside in freshwater, particularly salmonids (Gauldie 1996; Bowen et al. 1999; Melancon et al. 2005). Because of their relatively small atomic radii, Mg and Mn (0.86 and 0.81 Å, respectively) may be preferentially incorporated into otoliths comprised of mostly vaterite (Melancon et al. 2005). These elements may prove to be very useful when determining the environmental history of fish that have vateritic otoliths.

Elemental uptake at low temperature

In our study, otolith Ba:Ca and Sr:Ca levels did not follow a predictable pattern for fish reared at 10 °C. For these fish, otolith Ba:Ca and Sr:Ca were often higher for fish reared in baseline ambient concentrations than those from fish reared under low elemental concentrations, a pattern that was not detected in the 15 °C and 20 °C temperature treatments. Also, differences among tanks at this temperature, while not having a large effect on overall patterns, apparently drove the significance of tank effects in several tests. One of the original underlying bases for the relationship between otolith elemental uptake and temperature was presumed to be a kinetic (i.e., crystallographic) effect, similar to that of aragonictic corals. However, a meta-analysis of published laboratory studies published by Campana (1999) indicated that temperature-dependent Sr:Ca fractionation of otoliths is not a generalized phenomenon across taxa. Although the process of otolith elemental uptake is not currently well understood, the crystallization process is believed to be controlled by the rate of protein formation in the matrix. Protein formation in the otolith matrix is ultimately controlled by the rate of protein synthesis in the fish and, therefore, highly correlated with the growth rate of an individual (process reviewed by Campana 1999). Thus, it has been hypothesized that apparent temperature influences on otolith Sr:Ca are confounded with the physiological effects of protein synthesis and growth rate.

To determine whether the unusual results for fish reared at low temperature in the present experiment was consistent with physiological or kinetic effects, we conducted an additional statistical analysis to determine how growth rate and temperature interact to influence Sr:Ca in yellow perch otoliths. We began by selecting individuals in the 0 to 25th (slow-growing individuals) and 75th to 100th (fast-growing individuals) percentiles of total length for a given temperature treatment, removing all intermediate fish from the data set. We then analyzed the reduced data set using a two-way ANOVA with otolith Sr:Ca as a dependent variable and temperature, growth rate (fast and slow growth), and their interactions as independent variables. From this statistical analysis, both temperature ($F = 8.449$, df = 2, $P = 0.0004$) and growth rate ($F = 18.171$, df = 1, $P < 0.0001$) had a significant influence on Sr:Ca from yellow perch otoliths, whereas the interaction ($F = 1.527$, df = 2, $P = 0.221$) did not significantly influence Sr uptake. Sr:Ca was highest at 20 and decreased with decreasing temperature. Within a temperature treatment, Sr:Ca was higher in slow-growing individuals than in those with faster growth rates. Unfortunately, our results were inconclusive, providing support for both a physiological and a kinetic effect on Sr uptake in yellow perch otoliths. It should be noted that the temperature range in the present study is well below the temperature for maximum growth for yellow perch (23 °C; Hokanson 1977), and accordingly, our experimental fish likely grew at a level well below maximum rates. However, the range of temperatures in our experiment was selected to bracket the range of temperature that yellow perch will experience during early life stages. Indeed, water temperatures at or below 10 °C are common during the larval and juvenile stages of yellow perch and other percids (and salmonids) in the Great Lakes region. Therefore, field-based otolith microchemistry using early life stages of these fish will need to accommodate for the effect of low temperature on elemental uptake rates. Our results suggest that water temperature should be considered during any attempts to characterize differences in local populations using otolith microchemistry in the Great Lakes region.

Acknowledgements

The authors thank Mort Pugh and all of the Ohio Division of Wildlife hatchery personnel for providing fish for our experiments. Tyler Groll, Wes Eaken, and Jessica Best provided technical help by rearing the fish and maintaining treatment conditions over the course of the experiments. Anthony Lutton provided technical advice and assistance on the LA-ICPMS analyses. Financial support for this research was provided by a NOAA Interjurisdictional Fisheries Grant (NA04NMF4070371) and a Lake Erie Protection Fund Grant (LEPF 04-15).

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Appendix A

Table A1. Conditions in experimental tanks during juvenile yellow perch (*Perca flavescens*) experiments.

Treatment	Temperature (°C)	Tank	Year	Ba:Ca (mmol·mol^{-1})	Mg:Ca (mmol·mol^{-1})	Sr:Ca (mmol·mol^{-1})	TL	*n*
Baseline	10	9	2005	4.58±0.69	490.15±36.51	3.67±1.60	36.25±1.39	8
Baseline	10	12	2005	4.36±0.58	492.99±28.82	2.89±0.06	37.89±1.13	8
Baseline	10	18	2006	4.82±0.75	543.50±41.49	3.88±3.55	52.35±9.92	17
Baseline	15	6	2005	4.61±0.77	499.51±27.31	2.53±0.05	42.27±2.61	11
Baseline	15	7	2005	4.47±0.70	491.42±30.55	2.84±0.06	42.25±1.60	12
Baseline	15	16	2006	5.33±0.91	542.22±39.55	3.88±3.56	57.12±10.18	34
Baseline	20	2	2005	4.71±0.73	511.61±46.64	3.17±2.82	53.00±7.82	9
Baseline	20	4	2005	4.86±0.50	500.41±26.04	2.60±0.07	48.71±2.69	7
Baseline	20	14	2006	5.42±0.66	532.61±21.70	3.11±0.14	59.81±11.30	16
Low	10	17	2006	5.91±0.77	609.00±22.18	9.93±0.68	49.04±7.89	26
Low	15	15	2006	6.14±0.89	604.73±22.94	9.50±1.44	54.12±9.70	25
Low	20	13	2006	5.98±1.38	602.66±24.39	9.62±0.87	59.36±11.18	25
High	10	10	2005	7.37±3.45	689.38±113.57	16.42±0.78	36.70±1.70	10
High	10	11	2005	6.28±0.74	654.31±34.45	15.74±0.39	36.56±1.81	9
High	15	5	2005	6.40±0.59	665.23±31.82	15.80±0.30	42.70±2.58	10
High	15	8	2005	6.29±0.98	653.87±29.68	16.33±0.46	43.09±5.19	11
High	20	1	2005	5.41±1.28	673.81±29.75	15.18±0.97	50.13±6.77	8
High	20	3	2005	6.23±0.82	669.05±30.51	15.72±0.42	47.11±4.22	9

Note: Mean (± standard deviation, SD) Ba:Ca, Mg:Ca, and Sr:Ca water concentrations for each tank are provided with mean total length (TL, ±1 SD) and sample size (*n*).

PERSPECTIVE / PERSPECTIVE

Do riparian zones qualify as critical habitat for endangered freshwater fishes?

John S. Richardson, Eric Taylor, Dolph Schluter, Mike Pearson, and Todd Hatfield

Abstract: Identification of critical habitat is a key step in conservation and recovery of endangered and threatened freshwater fish. Critical habitat under Canadian and US legislation may include habitat that is not directly used by listed fish, provided it is necessary for species conservation or recovery. Riparian habitat meets biological criteria for critical habitat because riparian zones are integral to aquatic ecosystem functions of importance to many fish species and other organisms. These functions include provision of shade for temperature-sensitive species, control of channel complexity and sediment inputs through bank stabilization, input of large wood and allochthonous energy sources, and filtering of nutrients and toxins from adjacent land. In response to decades of stream-riparian research, widespread implementation of regulations to protect riparian zones in most developed countries represent a de facto consensus that riparian buffers are essential for aquatic ecosystem health and the maintenance of populations of fish and other species. Consistent with widespread riparian regulations deemed necessary to protect not-at-risk species, riparian habitat adjacent to a body of water containing a listed freshwater species should be considered biologically critical unless the habitat requirements of individual taxa are demonstrated to be insensitive to the ecological functions associated with riparian habitat.

Résumé : L'identification de l'habitat critique est une étape essentielle dans la conservation et la récupération des poissons d'eau douce menacés et en voie de disparation. Dans les législations canadienne et américaine, l'habitat critique peut inclure des milieux qui ne sont pas directement utilisés par les poissons concernés, à la condition qu'ils soient nécessaires pour la conservation ou la récupération de ces espèces. Les habitats riverains possèdent les critères biologiques d'habitats critiques parce que les zones riveraines sont nécessaires pour assurer des fonctions de l'écosystème aquatique d'importance pour plusieurs espèces de poissons et pour d'autres organismes. Ces fonctions incluent la production d'ombre pour les espèces sensibles à la température, le contrôle de la complexité du chenal et des apports de sédiments par la stabilisation des rives, l'apport de sources d'énergie allochtones et de débris ligneux de grande taille et la filtration des nutriments et des toxines provenant des terres adjacentes. À la suite de décennies de recherche sur la relation entre les cours d'eau et la zone riveraine, la mise en application très commune dans la plupart des pays développés de règlements pour protéger les zones riveraines représente un consensus de facto reconnaissant que les zones tampons riveraines sont essentielles à la santé des écosystèmes aquatiques et le maintien des populations de poissons et d'autres espèces. En accord avec les règlements largement répandus sur les zones riveraines jugées nécessaires pour la protection des espèces non vulnérables, tout habitat riverain adjacent à un milieu aquatique contenant une espèce d'eau douce figurant sur la liste des espèces en péril devrait être considéré comme biologiquement critique, à moins qu'on démontre que les besoins d'habitat de ce taxon particulier sont insensibles aux fonctions écologiques associées à l'habitat riverain.

[Traduit par la Rédaction]

Introduction

Canada's new legislation to protect endangered species, the Species At Risk Act (SARA; Government of Canada 2002), has been in place since 2002. As with most nascent environmental legislation designed to protect endangered species, there is confusion and controversy over aspects of both science and implementation. The most controversial steps are the listing process (Mooers et al. 2007; Hutchings and Festa-Bianchet 2009) and the identification of critical

Received 5 March 2010. Accepted 30 April 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 25 June 2010.
J21700

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doi:10.1139/F10-063

habitat for a listed species (Rosenfeld and Hatfield 2006). The decision to list is obviously pivotal because it determines which species will be subject to legal protection (Mooers et al. 2007). Once listed, decisions regarding critical habitat are equally far-reaching, because they establish the extent, attributes, and location of protected habitat and strongly influence habitat management, socio-economic impacts (e.g., Jones et al. 2006), and compensation to affected parties (e.g., Takekawa and Beissinger 1989; Kautz and Cox 2001).

Because spatial location and attributes of critical habitat profoundly affect species' persistence and the potential for socio-economic impacts, definitions of critical habitat for a listed species need to be (*i*) technically sound (i.e., firmly based in science and the biology of the focal species, so that definitions will ensure species persistence) and (*ii*) consistent with the criteria for critical habitat set out in legislation. In the following discussion, we briefly consider whether riparian habitat meets technical and biological criteria for critical habitat as applied to freshwater fishes and other listed aquatic organisms in lakes and streams (where riparian habitat refers to the vegetated zone adjacent to a water body; for detailed definitions, see Gregory et al. 1991, Naiman et al. 2005, and Richardson et al. 2005). This issue is topical because it impacts dozens of species and thousands of kilometres of aquatic and riparian habitat, has widespread implications for the conservation and management of listed freshwater fishes throughout Canada and the US, and has the potential for significant socio-economic impacts in many jurisdictions (e.g., Jones et al. 2006; but for economic benefits of riparian restoration, see Theurer et al. 1985). We recognize that areas well beyond the riparian zone also exert strong controls on aquatic habitat of listed species through effects on hydrology, water quality, and geomorphology (Harding et al. 1998; Kreutzweiser et al. 2008), and that broader protection may be required to maintain desired ecosystem attributes in many situations (e.g., Saunders et al. 2002; Abell et al. 2007; Nel et al. 2009). However, in this paper, we consider only the relevance of the riparian zone for conservation of species at risk and argue that there is scientific consensus on the dependence of aquatic ecosystems on the integrity of their riparian areas. Our goal is not to duplicate existing reviews of riparian function (e.g., Pusey and Arthington 2003; Naiman et al. 2005; Richardson and Danehy 2007), but rather to evaluate the significance of the riparian zone to the conservation of endangered freshwater species, an important conservation issue that has received little attention in the primary literature.

Critical habitat under SARA is defined as "… the habitat that is necessary for the survival or recovery of a listed wildlife species, and that is identified as the species' critical habitat in the recovery strategy or in an action plan for the species" and may include "… spawning grounds and nursery, rearing, food supply, migration, and any other areas on which aquatic species depend directly or indirectly [our emphasis] in order to carry out their life processes …" (Government of Canada 2002, s. 2(1)). (For the purpose of the following discussion, we interpret survival to represent long-term persistence of at least a minimum viable population and recovery to represent achievement of a population recovery target, which may equal or exceed the population size required for survival or persistence.) It is noteworthy that the above definition explicitly allows for the identification of habitat as critical even when it is not directly used by a species. This is especially relevant to species in freshwater habitats, where water quality and physical habitat structure are strongly influenced by activities on land (Harding et al. 1998; Abell et al. 2007; Nel et al. 2009).

Critical habitat under the US Endangered Species Act (ESA) is also broadly defined as "… the specific areas within the geographical area occupied by the species … on which are found those physical or biological features essential to the conservation of the species." and "... specific areas outside the geographical area occupied by the species if the agency determines that the area itself is essential for conservation" (Endangered Species Act of 1973; United States Congress 1973). This definition is also explicit in permitting areas that indirectly affect a species to be identified as critical, provided that a clear case can be made that it is "essential for conservation", i.e., it is not required that the habitat be directly occupied. It therefore follows that even though riparian habitat is not directly used by aquatic species, it meets the general technical criteria for critical habitat of aquatic species under both Canadian and US legislation, provided that a sound technical and biological case can be made that it is necessary habitat for species conservation and recovery. In contrast with Canadian legislation, US legislation explicitly allows the assessment of critical habitat to include consideration of socio-economic impacts. Regulators in the US have identified riparian areas as critical habitat for some species (e.g., Gila Chub; US Fish and Wildlife Service 2005) but not others (e.g., National Oceanic and Atmospheric Administration (NOAA) 2005, 2009), while clearly acknowledging that unoccupied upland terrestrial habitat qualifies as critical for aquatic species (e.g., NOAA 2000, 2005).

Does riparian habitat meet biological criteria for critical habitat of freshwater fishes?

We argue that an objective biological definition of critical habitat exists based on the habitat required for species persistence or to meet a recovery target (e.g., Rosenfeld and Hatfield 2006; Richardson and Thompson 2009). Based on the assumption of population limitation by habitat quantity and quality, Rosenfeld and Hatfield (2006) proposed simple screening criteria for evaluating whether habitat features were likely to be biologically critical to species persistence. These criteria include (*i*) habitat that is necessary to maintain ecosystem integrity and function or (*ii*) habitat that is disproportionately important (either among or within different habitat types) the singular or cumulative loss of which will result in significant population-level effects (for a recovered or recovering population) and (*iii*) the set or subset of habitats required for a species or population to persist (or achieve a recovery target). Note that candidate habitats do not have to meet all of these criteria to qualify as critical, and habitat that does not meet these criteria may be critical for reasons other than population limitation (see Rosenfeld and Hatfield 2006, their appendix A-5). Below we briefly consider whether the attributes of riparian habitat meet these

Table 1. Application of criteria for assessing whether aquatic critical habitat is sensitive to riparian function for selected aquatic taxa.

	Salmon–trout	Nooksack dace	Stickleback species pairs	White sturgeon	Deepwater sculpin
(1) Is in-stream critical habitat quality affected by riparian function?					
In-stream (or in-lake) habitat attribute					
Bank stability	Y	Y	—	—	—
Sedimentation	Y	Y	?	—	—
Terrestrial prey and organic matter input	Y	—	?	—	—
Aquatic prey production	Y	Y	—	—	—
Large wood and complex habitat	Y	—	—	—	—
(2) Is water quality affected by impaired riparian function in a way that could compromise species persistence?					
Water quality attribute					
Temperature	Y[a]	Y	—	—	—
Water clarity or turbidity	—	—	Y[b]	—	—

Note: Salmon–trout, generalized salmonids; Nooksack dace, *Rhinichthys* sp.; stickleback species pairs, *Gasterosteus* sp.; white sturgeon, *Acipenser transmontanus*; deepwater sculpin, *Myoxocephalus thompsonii*. Y indicates that a functional attribute is required for persistence of a species, a question mark (?) indicates uncertainty in requirement, and a dash (—) indicates that the function associated with the riparian zone may not be required for species' persistence.

[a] In streams where temperature is near an upper threshold.

[b] Decreased water clarity could trigger hybridization through impaired mate recognition (see text).

criteria in the case of freshwater bodies containing listed species.

First, is riparian habitat necessary to maintain aquatic ecosystem integrity and function? A very large body of science has developed over the past five decades documenting the importance of riparian zones to the health of freshwater ecosystems (Forest Ecosystem Management Assessment Team (FEMAT) 1993; Pusey and Arthington 2003; Naiman et al. 2005). Riparian zones provide a variety of functions, ranging from prevention of excessive stream bank erosion (Hassan et al. 2005) to maintenance of channel structure (Murphy and Koski 1989; Montgomery et al. 1995; Sweeney et al. 2004), shading of streams (Kiffney et al. 2003; Pusey and Arthington 2003; Moore et al. 2005), filtration of nutrients and toxins from adjacent agricultural fields (e.g., Barling and Moore 1994; Martin et al. 1999; Gay et al. 2006), protection from livestock grazing (Armour et al. 1994), and provision of allochthonous energy subsidies and a supply of large wood from riparian trees (Murphy 1995; Allan et al. 2003; Richardson et al. 2010). Riparian processes affect habitat attributes of importance to lake-dwelling fish (e.g., Christensen et al. 1996; Pace et al. 2004; Roth et al. 2007), as well as fish in rivers and streams, although impacts of riparian processes on physical habitat structure may be more pronounced in streams. Removal or disturbance of riparian vegetation can also facilitate the invasion of terrestrial and aquatic alien species (Pusey and Arthington 2003), which is a major factor in the decline of aquatic species at risk (Dextrase and Mandrak 2006). Moreover, stream and riparian systems are strongly linked by cross-ecosystem subsidies of resources flowing in both directions (e.g., Sabo and Power 2002; Knight et al. 2005; Richardson et al. 2010) and other processes that make it impossible to consider each subsystem in isolation.

This evidence is summarized in many reviews of riparian function (e.g., Gregory et al. 1991; Pusey and Arthington 2003; Naiman et al. 2005), as well as numerous studies documenting the effects of riparian alteration on fish and invertebrate community structure (e.g., Jones et al. 1999; Lorion and Kennedy 2009*a*, 2009*b*). Evidence for the negative effects of riparian forest removal on aquatic biota and ecosystems has been so overwhelming that most jurisdictions in the developed world have enacted legislation to protect riparian habitat (e.g., Blinn and Kilgore 2001; Lee et al. 2004; B.C. Ministry of the Environment 2005), with the primary goal of maintaining the health of aquatic ecosystems including populations of nonlisted biota, despite the significant costs of doing so (e.g., Jones et al. 2006). This legislation represents a de facto, multijurisdictional consensus on the importance of the riparian zone to aquatic species and reflects the strength of the science on which it is based. The only aspect up for debate is the exact width of buffer required for any particular water body or function (Kiffney et al. 2003; Marczak et al. 2010), which may depend on the ecology of the focal species.

Because the habitat requirements of species differ, consideration of riparian habitat as critical should depend on the effect that riparian habitat has on the quality of in-stream habitat for the species in question. It is possible that riparian habitat will be critical for some species but not for others. For example, riparian habitat may not be critical for a warm-water species that (*i*) is tolerant of siltation and turbid water, (*ii*) does not require clean gravel for egg incubation, and (*iii*) is only minimally dependent on allochthonous energy inputs. The humpback chub (*Gila cypha*; Minckley and Deacon 1991), a warm-water species adapted to the turbid waters of the Colorado River where riparian vegetation is naturally poorly developed, may be one example where riparian habitat is not an important component of critical habitat. However, fish species with habitat requirements that are unaffected by the functions associated with riparian vegetation are uncommon. It is much more common that riparian habitat plays a crucial general role in maintaining aquatic ecosystem function and health (Barling and Moore 1994; Murphy 1995; Pusey and Arthington 2003), as evidenced by the prescriptive nature of regulated riparian buffers, i.e., fixed widths rather than taxon- and site-specific widths. As a result, the default assumption should be that riparian habitat is necessary for the maintenance of proper ecosystem function and therefore is biologically critical for water bodies containing listed species. Application of this assessment should be abandoned only when there is clear evidence to the contrary for any specific taxon. Given the widespread regulatory requirement for riparian buffers on fish-bearing streams that harbour nonlisted species, to consider riparian habitat as not critical for the persistence of listed species would implicitly afford listed species less protection than taxa that are deemed not at risk.

The second criterion for biological significance is whether riparian habitat is disproportionately important relative to other available habitats and whether its loss can result in significant population-level effects. One of the core principles of critical habitat protection is that some habitats may be extremely important for species persistence, whereas others may be lost or degraded with minimal population-level effects. In some cases, it may be necessary to protect all of the available habitat for a species to ensure persistence, for instance, the hot water *Physa* that exists in a single hot spring (Te and Clarke 1985) and stickleback species pairs with global distribution restricted to a single lake (Hatfield 2009). However, in cases where habitat limitation is less extreme or less sensitive to disturbance, it may not be necessary to protect an entire drainage basin, and species' persistence can be ensured by identifying and protecting the most functionally important habitats. While the general functional importance of riparian habitat is well established (e.g., Gregory et al. 1991; Naiman et al. 2005; Richardson and Danehy 2007), its significance relative to other habitats in a drainage basin can be understood by considering the population-level effects of removing a riparian buffer adjacent to a stream relative to the effects of removing an equivalent area of forest distant from the water body. Although some upland terrestrial habitats in a drainage basin may be disproportionately important to aquatic ecosystem function (i.e., areas that are critical for slope stability or groundwater recharge), it is clear that in most cases, removing an upland strip of forest will have a much smaller impact on aquatic ecosystem function than removing an equivalent area adjacent to a water body.

The disproportionate importance of the riparian zone relative to other terrestrial habitats suggests that riparian habitat also meets the third biological criterion for critical habitat: that riparian habitat is part of the subset of habitats required for a species or population to persist (or achieve a population recovery target). As noted earlier, there is a general consensus in the scientific literature that an intact riparian zone is required to maintain normal aquatic ecosystem function and healthy fish populations. This consensus is manifest in both the near-universal riparian protection regulations afforded to fish-bearing streams in developed countries and the consistent focus on riparian restoration to rehabilitate degraded aquatic ecosystems (e.g., Beechie and Bolton 1999; Bernhardt et al. 2005), including the active restoration of riparian zones to aid recovery of many listed aquatic species (e.g., Hyatt et al. 2004; Pearson et al. 2008). Ultimately, the most definitive test of the importance of riparian forest to species persistence is to remove it in a controlled experiment and to document the effects on population abundance and persistence. In most cases, the rarity of species at risk, ethical issues, financial and logistic constraints, and the necessity to act quickly to prevent extinction preclude this type of experiment. However, in many instances, the experiment has already taken place, albeit without controls, and it is clear that removal of riparian forest (along with a suite of other habitat impacts, e.g., Findlay et al. 2001; Kerr and Cihlar 2004) is typically associated with the decline or endangerment of many taxa (e.g., Williams et al. 1993; Pess et al. 2002; Pearson 2007). In general, evidence is strong that degradation or removal of riparian vegetation can contribute to population declines that put species at greater risk because of smaller population sizes (Caughley 1994) and lower intrinsic rates of population increase in degraded habitats (Rosenfeld and Hatfield 2006). This supports the general conclusion that riparian habitat is part of the subset of overall habitats required for species persistence. Population viability analysis (PVA) is another approach for assessing the effects of habitat change on extinction risk (Akçakaya 2000; Haight et al. 2002), but the precise effects of riparian loss on vital rates (e.g., survival, growth, and fecundity) are inadequately parameterized for most listed species. In the absence of this information, explicitly considering whether loss of riparian function would degrade aspects of critical in-stream habitat quality is a useful interim criterion for evaluating the importance of riparian habitat to any particular taxon (Table 1).

We now briefly consider application of this logic to selected taxa (Table 1). We consider as examples a generalized salmonid and four endangered fish taxa that occupy distinctly different habitats: Nooksack dace (*Rhinichthys* sp.), a small fluvial cyprinid; stickleback species pairs (*Gasterosteus* sp.), which are found in a limited number of small coastal lakes in British Columbia; white sturgeon (*Acipenser transmontanus*), a large-river species native to British Columbia and the US Pacific Northwest; and the deepwater sculpin (*Myoxocephalus thompsonii*), which is endemic to deep cold-water lakes (Scott and Crossman 1973).

Most in-stream habitat attributes of importance to salmonids are sensitive to loss of riparian function, ranging from the negative effects of sedimentation on gravel interstices used for egg incubation to loss of pool habitat created by large wood inputs (Table 1). The other end of the sensitivity continuum is bracketed by deepwater sculpin. Although the detailed habitat requirements of deepwater sculpin are poorly understood, as a profundal benthic invertivore found at depths greater than 20 m in large cold-water lakes (e.g., Great Bear and the Laurentian Great Lakes; Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006), the ecology and persistence of deepwater sculpin should be relatively insensitive to riparian function. The three other listed species fall between these extremes of sensitivity (Table 1). Nooksack dace is a riffle-dwelling cyprinid with a limited distribution in southern British Columbia, where it is native to streams subject to a variety of habitat impacts associated with urbanization and agriculture. Because juvenile and adult Nooksack dace spawn and live in riffles, where they forage interstitially on benthic invertebrates, in-stream riffle habitat has been identified as biologically critical (Pearson 2007). Although there is no research on sediment impacts specific to Nooksack dace, abundant research on ecologically similar benthic insectivores (e.g., Mebane 2001) has demonstrated severe negative effects of sediment inputs through infilling of coarse substrate interstices, leading to decreased invertebrate prey abundance (Wood and Armitage 1997; Thompson et al. 2001; Suttle et al. 2004) and decreased interstitial habitat for benthic fish (for a more detailed development of these arguments, see Pearson 2007). Although riparian functions associated with provision of shade and allochthonous inputs are likely also important to dace, control of bank erosion leading to excessive sediment inputs to riffle habitat is probably the most important function of riparian vegetation that qualifies it as critical terrestrial habitat for protecting critical in-stream riffle habitat for Nooksack dace. It may also be an asset to conservation of this species that riparian areas can reduce flux of other contaminants as well, including nutrients and pesticides.

Stickleback species pairs, in contrast, are lake-dwelling limnetic and benthic-feeding invertivores that have locally co-evolved to feed on contrasting pelagic and benthic resources. Reproductive isolation between these recently evolved species is somewhat fragile (Kraak et al. 2001; Taylor et al. 2006) and is maintained by assortative mating based on differences in body size, male colouration, and possibly nesting microhabitat. Recently evolved fish species are sensitive to hybridization due to habitat changes such as increased turbidity that can interfere with mate recognition, which has been associated with hybridization and collapse of cichlid species in Lake Victoria (Seehausen et al. 1997). Two of five listed stickleback species pairs in Canada have already become extinct, and similar concerns about the effects of habitat change on hybridization apply to the remaining pairs. In contrast with Nooksack dace, it is a concern for stickleback species pairs that alteration or degradation of riparian habitat leads to an increase in turbidity or other habitat change, elevating the rate of hybridization, which is the primary reason for recommending the riparian zone as biologically critical for stickleback species pairs (Hatfield 2009).

White sturgeon is the largest freshwater fish in North America. Adults occupy a range of deepwater mainstem habitats in large rivers; habitat use by young juveniles can include low-velocity habitats such as side channels, tributary confluences, backwaters and sloughs (Bennett et al. 2005) or deep, low-velocity mainstem habitats and lake habitat (RL&L Environmental Services Ltd. 2000; Neufeld and Spence 2002; Golder Associates Ltd. 2003). Although riparian vegetation and large wood of riparian origin may have considerable influence on the channel and floodplain structure of medium to large rivers (Latterell et al. 2006; Latterell and Naiman 2007; Naiman et al. 2010), at present, there is no clear evidence that modification of the riparian zone will degrade in-stream critical habitat attributes for the listed populations of white sturgeon or decrease their probability of persistence (Table 1); consequently, recovery teams in the USA and Canada did not identify riparian habitat as biologically critical (US Fish and Wildlife Service 1999; National Recovery Team for White Sturgeon 2009).

In summary, we conclude that consistent with existing science and widespread riparian regulations, riparian habitat should be considered biologically critical for most species of freshwater fish, unless the habitat requirements of individual species indicate insensitivity to the ecological functions associated with riparian zones. The appropriate width of riparian buffer may be species- and site-specific and is best evaluated based on the biology of individual species using locally developed science or generic riparian regulations that are appropriate to the mature riparian plant community associated with a particular region, site, and water body. Although we have focused on the critical role of riparian habitat in this paper, we note that protecting the riparian zone may not in itself be sufficient to maintain stream ecosystem integrity (Roth et al. 1996; Roy et al. 2006; Kreutzweiser et al. 2008) or species at risk (Wenger et al. 2008) if development throughout a watershed (e.g., agriculture or urbanization) significantly alters hydrology or water quality. When a watershed is subject to extensive land use that may degrade in-stream habitat quality, sensitive aquatic species will persist only through careful management of development activities, and in some circumstances, larger areas of the drainage basin beyond the riparian zone may warrant consideration as biologically critical.

Finally, we suggest that scientists tasked with defining the biological attributes and spatial distribution of critical habitat for a particular species (in the biological rather than legal sense) should not base their assessments on whether habitat is protected by existing legislation or management options. While the suite of existing management tools and options available for habitat protection is an important consideration for implementing species recovery, it is irrelevant to the biological identification of critical habitat, which should be based on scientific principles and the biology of the species. Existing protection of riparian habitat through municipal, provincial, state, or federal legislation does not make riparian habitat any less biologically critical, although existing protection may minimize the socio-economic impacts of legal critical habitat designation and implementation.

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Canadian Journal of FISHERIES AND AQUATIC SCIENCES

Journal canadien des SCIENCES HALIEUTIQUES ET AQUATIQUES

Volume 67, Number 7, July 2010

Volume 67, numéro 7, juillet 2010

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0706-652X(201007)67:7;1-4